The beneficial effect of plasma on isolated saline-perfused frog hearts, first described by Ringer in 1883 and confirmed by Clark in 1913, has led to widespread interest in the isolation of naturally-occurring positively inotropic substances from plasma and other body tissues.\(^1\-^7\) The isolation from mammalian plasma of a protein system which increases the contractile force of isolated frog hearts was described recently by Hajdu and Leonard.\(^1^8\) This protein system consists of three globulins, designated cardioglobulin A, B, and C by them, the simultaneous presence of all three globulins being apparently necessary for the maintenance of inotropic activity. Leonard and Hajdu associated certain types of cardiovascular abnormalities with variations in the activity of the plasma cardioglobulins.

The effect of plasma on the contractile activity of hearts isolated from a variety of poikilothermic animals other than frogs was investigated and described by Nayler and McCulloch\(^1^9\) who showed that the augmented contractions which followed the addition of plasma to perfused isolated toad hearts were associated with improved mechanical efficiency. Curtain and Nayler\(^1^1\) isolated from human plasma a substance which evokes a positive inotropic response in perfused isolated hearts of toads (Bufo marinus) and which has a molecular weight of only 4000 to 8000.

The present paper describes certain effects of this substance on preparations of cardiac muscle dissected from several species, as well as its action on intact rabbits.

**Methods**

**PREPARATION OF CARDIOACTIVE SUBSTANCE FROM PLASMA**

Plasma was obtained from normal healthy blood donors. The cardioactive substance was separated as described by Curtain and Nayler,\(^1^1\) except that steps involving lyophilisation were eliminated because it was found that these reduced biological activity in varying degrees. By shaking with dry dispersed 7\% cross-linked polyacrylamide gel the plasma was concentrated threefold before filtration on the dispersed 5\% cross-linked polyacrylamide gel. The concentrated plasma was separated from the gel in a Buchner funnel in an International centrifuge, model Pr2, operating at 2,000 rpm. After gel filtration, but before proceeding with the isolation of the cardioactive substance on the ion-exchange column as described previously,\(^1^1\) the crude low-molecular weight fraction was concentrated threefold by shaking with 7\% gel as described above. The final stock solution of cardioactive plasma substance, dissolved in 0.05 M NaCl solution, contained 4 to 8 fig of cardioactive substance per ml.\(^1^2\) To measure activity we used the changed amplitude of isotonic contractions of ventricles isolated from toads (Bufo marinus).\(^1^2\) The inotropic activity of each freshly prepared solution of cardioactive plasma substance was equated with the inotropic action of norepinephrine (Levophed, Winthrop Laboratories) and expressed in equivalents of fig norepinephrine per ml.

**STUDIES OF ISOMETRICALLY CONTRACTING HEART MUSCLE**

Dog, rabbit, and monkey (Macaca irus and M. mulatta) papillary muscles, and rat trabecular carnea were excised from freshly exsanguinated animals of either sex and immediately immersed in 30 ml of aerated Tyrode solution of the following composition, in mM: NaCl, 130.02; KCl, 5.63; CaCl\(_2\), 2.16; NaHCO\(_3\), 25.00; glucose, 11.10; NaH\(_2\)PO\(_4\), 9.10; sucrose 13.15, prepared...
from analytical reagent grade chemicals dissolved in all-glass distilled water. The Tyrode solution was aerated continuously with 95% O₂ + 5% CO₂ and kept at 37.0°C. Isometric conditions were maintained by applying a constant resting tension of 2.5 g.

Strips of toad ventricular muscle, approximately 1.5 mm thick and 15 mm long were dissected and immediately immersed in 30 ml of aerated modified Ringer solution of the following composition, in mM: NaCl, 115.0; KCl, 3.2; CaCl₂, 0.65; NaHCO₃, 20.6; glucose, 16.5, NaH₂PO₄, 3.0; MgSO₄, 1.2; prepared from analytical reagent grade chemicals dissolved in all-glass distilled water. The modified Ringer solution was aerated with 95% O₂ + 5% CO₂ and kept at 25°C. Isometric conditions were maintained as above.

Dog, rabbit, and monkey papillary muscles, and rat trabeculae carneae were stimulated with suprathreshold rectangular pulses of 10 msec duration delivered from a Tektronix pulse generator, type 161, at the rate of 80 pulses/min. Strips of toad ventricular muscle were similarly stimulated but at the rate of 6 pulses/min. The stimulating electrodes used were either massive Ag-AgCl (surface area approx 4 cm²) plate electrodes having a resistance of 50 to 100 kohms. The recording microelectrode was either side or glass microelectrodes filled with 3 M KCl and having a resistance of 50 to 100 kohms.

Isometric contractions were detected with an RCA 5734 transducer, displayed on a dual beam cathode ray oscilloscope, Tektronix type 502, and photographed directly. Preparations were equilibrated for at least 30 minutes prior to the addition of drugs or of the isolated cardioactive plasma substance.

Transmembrane resting and action potentials were recorded from isometrically contracting strips of toad ventricular muscle stimulated by means of a KCl-filled microelectrode. Transmembrane potentials were measured by the microelectrode technique of Ling and Gerard using glass microelectrodes filled with 3 M KCl and having a resistance of 30 to 50 Mohms. The validity of the measuring and recording apparatus was checked regularly by inserting a 100 mv signal between the recording microelectrode and earth. Action potentials were displayed on a Tektronix 502 oscilloscope and photographed. The stimulating and recording electrodes were placed approximately 1 cm apart.

Procedure

Using the preparations described above, contractions were recorded from equilibrated isometrically contracting preparations of dog, rabbit, and monkey papillary, rat trabeculae carneae, and toad ventricular muscle, first in the absence, and then in the presence of isolated cardioactive plasma substance. The solutions of cardioactive plasma substance were diluted in Tyrode or modified Ringer solution as required and added directly to the perfusion bath.

Transmembrane potentials were recorded from toad ventricular muscle before and after the cardioactive plasma substance was added. In each case potentials were recorded from at least six impalpements and the observed variations fell within the calculated experimental limits of accuracy (± 2 mv).

STUDIES OF INTACT ANIMALS

These experiments were performed on intact rabbits of either sex, weighing between 2 and 3 kg. The rabbits were anaesthetized by pentobarbitone sodium (Sagatal, May and Baker, Ltd) 65 mg intravenously for the first injection followed by another 65 mg intraperitoneally 15 minutes later and then 65 mg intraperitoneally at 90-minute intervals. The skin of the anterior midline of the neck and of the groin over the left femoral artery was locally anaesthetized, using 2% Xylocaine. Two rabbits were pretreated with reserpine (Serpasil, Ciba) 1 mg/kg body wt, for four and five days respectively before experimentation.

The left femoral artery, right carotid artery, and right and left jugular veins were isolated and polyethylene catheters inserted into each isolated blood vessel. The catheter in the left femoral artery was passed as far as the abdominal aorta and its distal end was connected through a two-way tap to a densitometer cuvette. In some preparations the right femoral artery was also catheterized, the catheter being passed into the abdominal aorta and its distal end connected to a Statham P23Db transducer, the output of which was amplified and recorded as described below. The catheters in the right carotid artery and right jugular vein had side holes in the free ends and were passed into the left and right ventricular cavities respectively. In some experiments the catheter in the right carotid artery was allowed to remain in the lumen of the artery only. The distal end of each of these catheters was connected to a Statham P23Db transducer, the outputs of which were fed into a linear carrier amplifier and recorded with a Cambridge galvanometer photographic recorder. The left jugular vein was catheterized to the level of the superior vena cava; drugs and dye were injected through this catheter.

Cardiac output was measured by a dye dilution technique. “Cardiogreen” (indocyanine green) was injected intravenously and the concentration of dye in the arterial blood measured by a Waters X C 250A densitometer cuvette.
The arterial blood was withdrawn through the catheter in the femoral artery by means of a constant suction pump, and the dye curve so obtained recorded on a Waters potentiometer recorder operating at a constant paper speed of 5 mm/sec.

Cardiac outputs were measured before and after the injection of the cardioactive plasma substance. Prior to this injection duplicate estimations of the cardiac output were made and, provided such determinations revealed a constant cardiac output, the output was redetermined approximately 10 seconds after the plasma substance was injected, this being at the height of the pressor response. The cardiac outputs were calculated using the "forward triangle method." The output recorded after injection of the cardioactive plasma substance was expressed as a percentage of the average control output.

Respiration was recorded by placing a mercury filled rubber strain gauge around the thorax. The strain gauge formed one arm of a Wheatstone bridge, the output of which was fed directly into the Cambridge galvanometer photographic recording system. Thus, right and left ventricular pressures and respiration could be recorded simultaneously.

**Procedure**

The actions of the cardioactive plasma substance on cardiac output, left and right ventricular pressures, respiration, and heart rate of intact rabbits were compared with those of bradykinin (Sandoz), angiotensin (Hypertensin, Ciba), norepinephrine and epinephrine (Hermette, D. Bull and Company). In addition, the actions of the isolated cardioactive substance on the previously reserpinized rabbits described above and on rabbits which were pretreated with the adrenergic blocking drugs, 2-N-p-tolyl-N-m-hydroxyphenylaminomethyl-imidazoline (phenolamine, Regitine, Ciba) and 1-(2-naph-thyl)-2-isopropylaminomethanol hydrochloride (pro-nethalol, Alderlin, ICI, 38174) were determined.

**Results**

Stock solution of cardioactive plasma substance in a dose of 0.1 ml containing 0.4 to 0.8 μg of cardioactive plasma substance, when added to isotonically contracting toad ventricles perfused with 3 ml Ringer solution, evoked a positive inotropic response approximately equal in magnitude to that produced in the same preparation by the addition of 2 μg norepinephrine. Figure 1 shows a typical response in which the contractions began to increase in amplitude immediately following the addition of the cardioactive substance. After the positive inotropic response elicited by the cardioactive plasma substance had developed fully, continued perfusion, without renewal of the perfusion fluid, resulted in the slow return of contractions to their original amplitude over a period of approximately 25 minutes. Repeated replacement of the perfusion fluid reduced this time interval to approximately 15 minutes. After the control amplitude of contraction was re-established, further additions of the cardioactive plasma substance produced further positive inotropic responses without evidence of tachyphylaxis. Solutions of isolated cardioactive plasma substance which had been stored at 4°C for seven days retained approximately 80% of their inotropic activity.

**Isometric Contractions**

The typical inotropic effect of 0.1 ml of the cardioactive plasma substance on dog and monkey isometrically contracting papillary muscles, bathed with 30 ml Tyrode solution, is shown in figure 2A and B. Such augmented
Contractions recorded from isometrically contracting (A and C, dog and B, monkey) papillary muscles immersed in Tyrode solution (small contraction) and in Tyrode solution containing cardioactive substance (large contraction, see text). Stimulation through Ag-AgCl plates, 80 pulses/min.

Contractions were obtained whether the muscle was stimulated by means of the Ag-AgCl plate electrodes or the KCl-filled microelectrodes described above. Therefore the increased tension, that developed after the cardioactive plasma substance was added, probably did not reflect recruitment of previously unresponsive fibres or increased conduction velocity.

The onset of the positive inotropic response was rapid, appearing a few seconds after the cardioactive plasma substance was added. The augmented contractions persisted for approximately 15 minutes after which they slowly returned to the control level. The addition of cardioactive plasma substance as above to isometrically contracting preparations of rabbit papillary, rat trabeculae carneae, and toad ventricular muscles resulted similarly in the development of increased tensions during contraction. Dog, rabbit, and monkey papillary muscle, and toad ventricular muscle, which had been treated previously for at least 30 minutes with 30 µg/ml of the β-adrenergic antagonist, pronethalol, and in which 10 to 20 µg/ml norepinephrine failed to elicit a positive inotropic response, responded to the addition of cardioactive plasma substance with augmented contractions.

Comparison of the isometric contractions, recorded before and after the cardioactive plasma substance was added to the preparations described above, showed that such augmented tensions were associated frequently with a decrease in the time required during contraction to develop peak tension. Such a response is displayed in figure 2A and B. In other similar preparations the positive inotropic effect of the cardioactive plasma substance developed apparently without any accompanying change in the time required to develop peak tension, as is shown in the isometric contractions of dog papillary muscle in figure 2C. The positive inotropic action of the cardioactive plasma substance was not associated with a prolongation either of the time required to develop peak tension during contraction, or of the total duration of contraction. Angiotensin (1 x 10⁻⁶ to 1 x 10⁻⁴M) failed to produce a positive inotropic response in toad ventricular muscle whereas the cardioactive plasma substance produced a marked positive inotropic response; the isometric contractions of toad sartorius muscle were not enhanced.
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The positive inotropic effect of the plasma cardioactive substance on toad ventricular muscle was accompanied by unchanged transmembrane resting potentials and thresholds of electrical excitability. Figure 3 shows action potentials recorded from a typical preparation of toad ventricle before (A) and three minutes after (B) the cardioactive plasma substance was added. The decreased duration of the action potential recorded after addition of plasma substance is attributable apparently to an increased rate of repolarization.

Studies on Intact Animals

Approximately three seconds after the intravenous injection of 0.02 ml of freshly prepared cardioactive plasma substance a marked rise in the left ventricular systolic blood pressure was noted, together with raised systolic and diastolic abdominal aortic and carotid artery pressures and, after a further seven seconds, with a raised left ventricular diastolic pressure. A less marked rise in the right ventricular systolic pressure was noted, the right ventricular diastolic pressure remaining unchanged. Figure 4 shows typical simultaneously recorded right and left ventricular pressure tracings, together with the respiratory rate, before and after the injection of 0.02 ml of cardioactive plasma substance.

The results from a typical cardiac output study are displayed in figure 5, which compares a control dye dilution curve with that recorded 10 seconds after cardioactive plasma substance was injected. Figure 5 shows also blood pressures recorded simultaneously in the left ventricle and the abdominal aorta. The results of eight other studies of cardiac output are summarized in table 1. In each of twelve experiments the intravenous injection of 0.02 to 0.1 ml of freshly isolated cardioactive plasma substance raised cardiac output and also increased systolic and diastolic pressures. In three experiments cardioactive plasma substance was used after storage at 4°C for 10 days. In these particular experiments the blood pressures remained unchanged following the intravenous injection of the plasma substance, although cardiac output was increased by 25%.

Similar intravenous injections of freshly prepared cardioactive plasma substance into previously reserpinized rabbits produced responses similar to those recorded from nonreserpinized rabbits. The pressor activity of the cardioactive plasma substance, as indicated by changed right carotid artery pressure, was not blocked in rabbits in which the pressor response to norepinephrine, and the depressor response to isoproterenol, had been blocked by prior treatment with phentolamine (3 μg/kg) and pronethalol (3 mg/kg).

The data from experiments in which the action of the cardioactive plasma substance on intact rabbits were compared with those of other known vaso-active substances, including epinephrine (3 μg/kg), norepinephrine (3 μg/kg), angiotensin (3 μg/kg) and bradykinin (3 μg/kg) are summarized in table 2. According to these data the effects of cardioactive plasma substance were clearly distinguishable from those of the polypeptides angiotensin and bradykinin.
Typical simultaneous records of right and left ventricular pressures and of respiratory rate from a rabbit before and after the intravenous injection of 0.02 ml cardioactive substance (C.A.S.). RV: right ventricular tracing; LV: left ventricular tracing; Sys.: systolic. Photographic recorder speed 2.5 mm/sec. Left ventricular diastolic levels are concealed in the right ventricular tracing.

Discussion

The present results confirm and extend previously reported observations relating to the isolation from mammalian plasma of a substance, of molecular weight 4000 to 8000, which elicits a positive inotropic response in isolated isotonically contracting toad ventricles. They show also that this substance produces a marked positive inotropic response from several types of mammalian cardiac muscle, including isometrically contracting papillary muscles isolated from dogs, rabbits, and monkeys, and trabecular carneae isolated from rats. The intravenous injection of this substance into anaesthetized rabbits raised systemic arterial pressures, raised right and left ventricular pressures, increased cardiac output and accelerated heart rates. Control studies showed...
that these effects were not attributable to the 0.05 M NaCl solution used for the preparation of stock solutions of cardioactive plasma substance or to the Tyrode and Ringer solutions with which dilutions were prepared. The positive inotropic action of 0.1 to 0.2 µg of isolated cardioactive plasma substance was found to be approximately equal to that of 3 µg norepinephrine, indicating that on a w/w basis, the activity of the cardioactive plasma substance is approximately ten times greater than that of norepinephrine.

It seems unlikely that the positive inotropic action of this substance can be attributed wholly to either the release of endogenously stored catecholamines from within the myocardium or to a changed distribution of the excitatory stimulus throughout the muscle mass. The positive inotropic effect was not blocked either by the β-adrenergic blocking drug, pronethalol, or the combination of this drug with phentolamine and it was obtained whether excitation was produced by means of the large plate electrodes or by a single KCl-filled microelectrode.

The altered duration of the action potentials recorded after the substance was added to toad ventricular muscle may mean that it changed the selective ionic permeability of cardiac cell membranes. It is impossible to determine from the presently available data whether or not such a changed selective ionic permeability is necessarily associated with the positive inotropic action. The rapidity of action points towards a surface-located site of action. When considering such a possibility it is useful to remember that the recently demonstrated continuity between the extracellular space and the sarcoplasmic reticulum in muscle\(^1\) may make it possible for a surface-located site of action to be distributed throughout the muscle mass and brought into reasonably close proximity to the contractile proteins themselves.

Many other cardioactive substances have been isolated previously from plasma. Such substances include the high molecular weight cardioglobulins A, B, and C isolated and characterized by Hajdu and Leonard.\(^8\) Leonard and Hajdu\(^9\) reported recently that the activity of this cardioglobulin system was greatly decreased in the presence of cut or damaged cells. During the present study the presence of such damaged cells, as were present in the thin isometrically contracting strips of toad ventricular or dog, rabbit, and monkey papillary muscle, apparently failed to inhibit the inotropic action of the substance being investigated.

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FIGURE 5

(A) Typical dye dilution curves recorded from a rabbit before (control) and 10 seconds after the intravenous injection of 0.02 ml of cardioactive substance (C.A.S.). Paper speed, 5 mm/sec. (B) Simultaneous records of left ventricular and abdominal aortic pressures of the same rabbit before and after the injection, at the arrow, of 0.02 ml cardioactive substance. Ao sys.: abdominal aortic systolic pressure; Ao dias.: abdominal aortic diastolic pressure; LV sys.: left ventricular systolic pressure; LV dias.: left ventricular diastolic pressure. Photographic recorder speed, 2.5 mm/sec.
TABLE 2

Effect of Cardioactive Plasma Substance, Norepinephrine, Epinephrine, Angiotensin, and Bradykinin on Left and Right Ventricular Pressures, Heart Rate, Cardiac Output, and Respiratory Rate in Anaesthetized Rabbit*

<table>
<thead>
<tr>
<th>Cardioactive plasma substance</th>
<th>LV Pressures</th>
<th>RV Pressures</th>
<th>Heart rate</th>
<th>Cardiac output</th>
<th>Respiratory rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Angiotensin</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*+ denotes an increase; — denotes a decrease; 0 denotes no change. LV denotes left ventricular pressure, before and after the injection of 3 μg/kg phenolamine and 3 mg/kg pronethalol. RV denotes right ventricular pressure.

Other cardioactive substances separated from plasma include the polypeptides angiotensin and bradykinin. The mode of action of the presently investigated cardioactive plasma substance appears to differ essentially from the action of either of these peptides. Angiotensin is a pressor drug which has been shown to evoke a marked positive inotropic response from isolated preparations of cat papillary muscle, this positive inotropic effect being associated with an unchanged duration of the time required during each contraction to develop peak tension. The positive inotropic activity of angiotensin therefore contrasts with that of the cardioactive substance described above.

Angiotensin has a biphasic effect on the circulation of intact animals. It initially impairs cardiac function, decreases cardiac output, and reduces the force of ventricular contraction, after which it produces a positive inotropic response associated with a raised cardiac output and force of ventricular contraction. These findings of Fowler and Holmes were confirmed during the present study and serve to differentiate between the mode of action of angiotensin and that of the cardioactive substance.

Bradykinin has been shown to increase cardiac output in intact animals; in addition, it reduces arterial blood pressure and therefore differs in its action from the substance investigated in the present experiments. Previously, it was shown that bradykinin failed to evoke a positive inotropic response from isolated toad ventricular muscle, a finding which was confirmed and extended to dog, monkey, and rabbit papillary muscle during the above experiments.

The experiments on intact animals showed consistently that the raised systemic blood pressure produced by the injection of freshly prepared cardioactive plasma substance was associated with an increased cardiac output. The failure of solutions of cardioactive plasma substance, which had been stored for ten days at 4°C, to elicit a pressor response in association with the raised cardiac output requires further investigation.

The physiological significance of the cardioactive plasma substance described above has yet to be elucidated. Its relationship, if any, to the higher molecular weight globulin system isolated by Hajdu and Leonard requires investigation. The isolation of such substances from plasma lends support to the concept that substances distributed in the circulating plasma may play a role in regulating the activity of cardiovascular muscle.

Summary

Some effects of a relatively low molecular weight substance, isolated from human plasma, on isolated amphibian and mammalian...
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cardiac muscle and on intact rabbits have been studied. This cardioactive plasma substance elicited a positive inotropic response from isolated isometrically contracting papillary muscle of rabbits, dogs, and monkeys, from trabeculae carneae of rats, and from ventricular muscle of toads. Its positive inotropic activity was associated with a shortened duration of action potentials. Studies on intact animals showed that the intravenous injection of this substance raised systemic pressures, raised right and left ventricular systolic pressures, and increased cardiac output. Adrenergic blocking drugs failed to block the inotropic and pressor effects of freshly isolated cardioactive plasma substance.

Acknowledgment

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References

1. Ringer, S.: A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart. J. Physiol. (London) 4: 29, 1883.
Some Properties of a Cardioactive Substance Isolated From Human Plasma
WINIFRED G. NAYLER, PETER G. C. ROBERTSON, JOCELYN M. PRICE and THOMAS E. LOWE

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