Effects of Hypertensin on Arterial Pressure, Heart Work and Cardiac Oxygen Utilization

By Ivan E. Forte, M.S., Louis Potgieter, Ch.B., and Jerry E. Schmitthenner, M.D.

With the assistance of A. Jane Williams, A.B., Hunter Neal, M.D., Toussef Shakashir, M.D., Robert Richards, M.D., and J. H. Hafkenschiel, M.D.

The purpose of the work was to investigate the effects of acute increases in left ventricular pressure work on coronary blood flow, myocardial oxygen and carbohydrate utilization. We were able at the same time to evaluate the application of the nitrous oxide method to the measurement of changes in coronary blood flow resulting from drug-induced changes in the pressure work.

A paired experimental design was used so that 2 consecutive periods of observation could be made on the same animal. The same type of study was then repeated at a later date with the same animal, the second control period being replaced by a period of drug infusion. We wished to test an experimental plan in which many observations were made on a few animals, each serving as its own control and each returning for several tests. The synthesis of Hypertensin II peptide (Hypertensin)* has made it possible to compare the pressor potential of this drug with the results of experiments performed using angiotensin, renin and Hypertensin I.1-2 At present, the synthetic Hypertensin appears to correspond completely with the naturally occurring angiotensin.3,4 This substance was tested since it might be the most potent pressor amine available and because it might eventually have more advantages and fewer disadvantages clinically than levarterenol.5-7 To us, Hypertensin was an agent which might increase left ventricular pressure work independently of any increase in left ventricular stroke work or heart rate.

Methods

The experimental design consisted of paired periods of observation for the purpose of comparing the changes in coronary blood flow and left ventricular work from the first to the second period, i.e., control-control. These differences were then compared with the changes observed in control-drug infusion experiments, using the same anesthesia in the same dog. The paired observations were made at intervals of approximately one month for any one dog.8-10 An identical procedure was used in all experiments, the time course being the same as shown in figure 1. This figure illustrates a Hypertensin experiment, 1 of 7 tests made in this same animal on different days.

Young healthy dogs were studied under the combination anesthesia morphine—"Dial"—urethane—pentobarbital—sodium (MDUP),* as recommended by Foltz.10

*The technic for the administration of the anesthesia is as follows: A dose of 2.0 mg./Kg. of morphine is given subcutaneously. Forty-five minutes later, a 0.3 ml./Kg. injection of pentobarbital—sodium mixed with "Dial"—urethane (0.15 cc. of 65 mg./cc. pentobarbital—sodium and 0.15 cc. of "Dial"—urethane is given intravenously. After 1 to 1½ hours, during which time the animal is operated on and catheterized, and approximately 30 minutes before the first observation period, a second dose of morphine in the amount of 0.5 mg./Kg. is administered intramuscularly. A third dose of 0.5 mg./Kg. concentration given about 30 minutes before the second period of observation brings the total dosage of morphine to 3.0 mg./Kg. of body weight.

From the Division of Research, Lankenau Hospital, Philadelphia, Pa.

Supported in part by a research grant (H1817) from the National Heart Institute, U.S. Public Health Service, and in part by grants from the Tobacco Industry Research Committee (QR17), and the Committee on Problems of Alcohol of the National Academy of Sciences-National Research Council (UV25), and the Heart Association of Southeastern Pennsylvania.

A preliminary report of this investigation was made by Dr. Potgieter on April 17, 1959, before the American Physiological Society at Atlantic City, N. J. (Fed. Proc. 18: 481, 1959).

Dr. Schmitthenner is a Postdoctoral Research Fellow of the National Heart Institute (HF6498) at Lankenau Hospital, Philadelphia, Pa., 1956 to 1958.

Received for publication May 27, 1960.

*Made available as Hypertensin—Ciba (BA19900A) by Dr. Albert Plummer of Ciba Pharmaceutical Products, Inc., Summit, N. J.
Figure 1
Mean arterial pressure (MAP) (mm. Hg), heart rate (HR) per minute, pulmonary artery pressure (PAP) (mm. Hg) with the systolic pressure shown as the top line and the diastolic pressure shown as the lower line, and respiratory minute volume (R.M.V.) (L./min.) were measured during a period of time indicated in minutes along the abscissa. The coronary blood flow (Cor. Flow) (cc/100 Gm. L.V./min.) was determined by the nitrous oxide desaturation method and the periods of time during which the measurement was made are shown, as well as the left ventricular work (L.V.W.) (Kg.M./min.) calculated for that same period.

During the paired control procedure, 0.9 per cent physiologic saline with 0.2 mg. per cent heparin was permitted to drip throughout the 2 periods. For the control part of the Hypertensin experiment a similar saline with heparin infusion was administered. This period was followed, after 20 to 30 minutes, by a 15-minute infusion of Hypertensin. The dosage of Hypertensin was selected after trial infusion of 1/3, 1 and 3 μg./Kg./min. had been tested and pressure responses established as being proportional to the dosage in this range. The drug was infused into a peripheral vein, using a constant rate infusion pump.

Arterial, mixed venous and coronary sinus blood samples necessary for measurements of coronary blood flow and cardiac output were drawn in the final 12 minutes when a relatively steady state had been established. An interval of 25 to 30 minutes between duplicate runs was allowed for saturation with nitrous oxide gas. We assumed that the coronary flow remained constant during its period of measurement. This assumption was probably not valid during the drug infusion periods. Also assumed to be unchanged during the time of the flow measurement were the cardiac arteriovenous metabolite differences.

Seven-hole, 3-way stopcock manifolds, on which lightly oiled, heparinized, 10-ml. syringes were mounted, were used to draw discontinuous blood samples for the nitrous oxide coronary blood flow determinations. Femoral artery and coronary sinus blood samples were taken at a rate of 1 ml. every 10 seconds. Six pairs of these arterio-venous samples were drawn for the coronary blood flow determination. The first pair was drawn during the last minute of inhalation of the 15 per cent nitrous oxide gas mixture. Compensation was made for the dead space in the sampling system. The other samples were collected during the first, second, third, fourth, and tenth minutes of nitrous oxide desaturation. The analysis of these samples served as a basis for plotting the nitrous oxide desaturation curves. Three 12-cc. samples were drawn over the fifth and sixth minutes of desaturation at the same rate of 1 cc. every 10 seconds from the femoral artery, coronary sinus and pulmonary artery.

These 12-cc. blood samples were used for the analysis of oxygen and carbon dioxide content,
## Table 1

### Observations of Five Control-to-Control Studies and Six Control-to-Hypertensin II Peptiden Studies on Four Dogs

<table>
<thead>
<tr>
<th>Observations</th>
<th>Initial mean</th>
<th>Difference mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 C*</td>
<td>6 C*</td>
</tr>
<tr>
<td>Coronary flow (cc/100 Gm. L.V./min.)</td>
<td>105</td>
<td>105</td>
</tr>
<tr>
<td>Cardiac oxygen uptake (cc./100 Gm. L.V./min.)</td>
<td>13.5</td>
<td>14.1</td>
</tr>
<tr>
<td>Left ventricular work (Kg. M./min.)</td>
<td>7.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Cardiac output (L/min.)</td>
<td>4.8</td>
<td>3.5</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm. Hg)</td>
<td>108</td>
<td>122</td>
</tr>
<tr>
<td>Cardiac rate per min.</td>
<td>78</td>
<td>83</td>
</tr>
<tr>
<td>Peripheral resistance (pressure + cardiac output)</td>
<td>1.25</td>
<td>1.75</td>
</tr>
<tr>
<td>Coronary resistance (pressure + coronary flow)</td>
<td>1.11</td>
<td>1.40</td>
</tr>
<tr>
<td>Cardiac efficiency (%)</td>
<td>22.7</td>
<td>21.6</td>
</tr>
<tr>
<td>Systemic arteriovenous oxygen</td>
<td>4.27</td>
<td>4.60</td>
</tr>
<tr>
<td>Arterial hemoglobin (Gm.)</td>
<td>14.8</td>
<td>14.9</td>
</tr>
<tr>
<td>Arterial hematocrit</td>
<td>49.3</td>
<td>49.4</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.26</td>
<td>7.25</td>
</tr>
<tr>
<td>Arterial pO₂ (mm. Hg)</td>
<td>57</td>
<td>60</td>
</tr>
<tr>
<td><strong>Oxygen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial O₂ content (cc./100 cc.)</td>
<td>17.81</td>
<td>17.94</td>
</tr>
<tr>
<td>Arterial O₂ saturation (%)</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>Coronary sinus pO₂ (mm. Hg)</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Coronary arteriovenous oxygen</td>
<td>12.63</td>
<td>13.48</td>
</tr>
<tr>
<td>Coronary O₂ extraction (%)</td>
<td>71.2</td>
<td>74.0</td>
</tr>
<tr>
<td>(A-V) O₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial O₂</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Symbols:

C: Mean observed value during initial control period of control-to-control studies.

C-H: Mean observed value during initial control period of control-to-Hypertensin studies.

C-C: Mean change from initial control period to second control period.

C-H: Mean change from initial control period to second Hypertensin period.

†p<0.01.

the pulmonary artery samples being necessary for the determination of the cardiac output. The same femoral artery and coronary sinus samples were also used for the determination of the hydrogen ion concentration with a Cambridge pH meter and for the determination of the hemoglobin concentrations with an Evelyn colorimeter. From these data, the oxygen tension was calculated, using the charts of Dill.11 Hematocrits were read after spinning the samples at 2,000 r.p.m. for 30 minutes. The oxygen tension of the blood was calculated from the derived coronary sinus venous oxygen, the arterial blood saturation and pH values. Utilizing the pH and carbon dioxide values, nomograms were employed to obtain the tension of carbon dioxide.12

One more pair of samples from the femoral artery and coronary sinus, drawn in uncoiled heparinized syringes during the seventh minute of desaturation, was used for the determination of glucose, lactate and pyruvate blood levels. Lactate levels were assessed by determining the lactic acid with a modified method based on that of Barker and Summerson, pyruvic acid according to the method of Friedmann and Haugen for blood pyruvate and blood glucose with the procedure as outlined by Hagedorn and Jensen with a modification by Somogyi.9

The expired air was collected from the second to the seventh minute of the desaturation period. The gas samples were analyzed for oxygen and carbon dioxide, using the method of Scholander.13 The nitrous oxide blood determinations for the Hypertensin results were done by the infrared absorption analysis method.14 The formula used was the general one applying the Fick principle to left ventricular blood flow and involved dividing the final venous nitrous oxide value by the sum of the A-V differences.

After the observations were made, the catheters
PERCENT CHANGE OF OBSERVATIONS
REPEATED 30 MINUTES LATER
Morphine dial urethane
pentobarbital
Anesthesia
alone
Hypertensin
□ pepliden
1μg/kg/min.

This bar graph shows the percent change from the first to the second period of observation under morphine-pentobarbital-urethane anesthesia. The data on cardiac output, mean arterial pressure, left ventricular work, coronary flow and cardiac oxygen metabolism are shown in the column on the right. The control-control studies are represented under "Anesthesia alone." The broken lines show the percent coefficients of variation (the ratio of the standard deviations of the individual differences to the initial values of the parameters). The upper portion of the figure includes cardiac output (CO), mean arterial pressure (MAP) and left ventricular work (LVW), and the lower portion shows coronary blood flow (CBF), cardiac arteriovenous oxygen difference (ΔA-VO₂), and oxygen consumption (O₂ cons.). See text.

Calculations
The various cardiodynamic and metabolic functions were calculated by applying the basic data, as has been previously reported. Cardiodynamics—glucose, lactate, pyruvate and oxygen—was calculated by multiplying its coronary arteriovenous difference by the coronary blood flow, thereby obtaining a value for uptake in μl./100 Gm. left ventricle/min. or, for oxygen, cc./100 Gm. of left ventricle/min. The per cent myocardial extraction was also determined for oxygen, lactate, pyruvate and glucose. Left ventricular mechanical efficiency was calculated as previously described.

All of the data acquired during the paired coronary flow determinations were considered on the basis of the mean change from the first to the second observation. The mean values of the changes from the first control to the second control were compared with the mean values of the changes from the control to the second control.
HYPERTENSIN ON CARDIAC METABOLISM

Table 2

Observations of Fire Control-to-Control Studies and Six Control-to-Hypertensin II Peptiden Studies on Four Dogs

<table>
<thead>
<tr>
<th>Observations</th>
<th>Initial means</th>
<th>Difference means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C*</td>
<td>Cn*</td>
</tr>
<tr>
<td>Pyruvate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial (mg. %)</td>
<td>0.69</td>
<td>0.77</td>
</tr>
<tr>
<td>Coronary A-V (mg. %)</td>
<td>0.29</td>
<td>0.30</td>
</tr>
<tr>
<td>Cardiac extraction (%)</td>
<td>34.8</td>
<td>34.5</td>
</tr>
<tr>
<td>A-V Art.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac uptake (mg./100 Gm. L.V./min.)</td>
<td>0.26</td>
<td>0.36</td>
</tr>
<tr>
<td>Lactate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial (mg. %)</td>
<td>8.1</td>
<td>7.7</td>
</tr>
<tr>
<td>Coronary A-V (mg. %)</td>
<td>2.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Cardiac extraction (%)</td>
<td>26.4</td>
<td>25.7</td>
</tr>
<tr>
<td>Cardiac uptake (mg./100 Gm. L.V./min.)</td>
<td>2.2</td>
<td>2.3</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial (mg. %)</td>
<td>73</td>
<td>79</td>
</tr>
<tr>
<td>Coronary A-V (mg. %)</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Cardiac extraction (%)</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Cardiac uptake (mg./100 Gm. L.V./min.)</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

*Symbols:
C: Mean observed value during initial control period of control-to-control studies.
Cn: Mean observed value during initial control period of control-to-Hypertensin studies.
C-C: Mean change from initial control period to second control period.
C-H: Mean change from initial control period to second Hypertensin period.
^p<0.01.

Results

This report analyzes observations made in 11 coronary blood flow experiments on 4 dogs. Six of these observations were made during control-Hypertensin experiments, and 5 were control-control tests.*

Hypertensin increased both mean arterial pressure (122 to 188 mm. Hg; figs. 1 and 2) and left ventricular work (5.9 to 8.1 Kg.M./min./100 Gm.; table 1). During the pressure increases induced by the drug, arterial hemoglobin, hematocrit, glucose and lactate levels increased in arterial blood. The increased cardiac lactate utilization was the only significant change in cardiac metabolism (table 2).

Discussion

The effects of Hypertensin in dogs under anesthesia are shown in figure 3 as the per cent change from the control observation. The components of left ventricular work are depicted in the top part of this bar graph.

*Data used in the statistical analysis of the observations performed in this laboratory on coronary blood flow, left ventricular weight, cardiac oxygen and carbohydrate moiety of uptake and left ventricular work for each experiment in 14 dogs have been tabulated (Tables A, B, C) and are deposited with the American Documentation Institute Auxiliary Publication Project. Tables A, B, and C have been deposited with the ADI as Document number 6345. A copy may be obtained by citing the number and by remitting $2.50 for photoprints or $1.75 for 35-mm. microfilm. Advance payment is required. Make checks or money orders payable to: Chief, Photoduplication Service, Library of Congress. Also the mean changes in all parameters measured during the varying anesthesia levels (control-control observations) and the mean changes observed during the drug infusions (control-drug observations) are available from the same Institute.
The dotted line shows the per cent coefficient of variation (the standard deviation of the individual differences divided by the initial value of the parameter $\times 100$). The pressure elevation and the work increase induced by Hypertensin are greater than the per cent coefficient of variation. In the bottom of the same figure are shown the components of cardiac oxygen consumption. The apparent greater coronary blood flow and cardiac oxygen utilization rate with Hypertensin were associated with the increased pressure work.

The percentage coefficient of variation of coronary blood flow was large in this small series of dogs. It was not possible to measure a significant increase in coronary flow during the acutely induced increases in pressure work.

These experiments showing the great variance in coronary blood flow (fig. 3) have convinced us of the difficulties involved in interpreting the results of paired observations in clinical experiments (using the nitrous oxide method) designed to compare the mean values of coronary blood flow and cardiac oxygen utilization during a drug action with the mean values observed initially.

The experiments do show that in healthy young dogs with adequate coronary arteries, the oxygen tension of the myocardial cells, as reflected in left ventricular venous blood draining into the coronary sinus, was maintained in a relatively low range, 11.4 to 30.6 mm. Hg. Coronary sinus oxygen tension was unchanged during the drug infusion. This lack of change implies that under these experimental conditions, Hypertensin has the ability either to open cardiac arteriovenous shunts or to increase coronary blood flow to meet the demand for increased myocardial oxygen. If the latter is true, then the findings support the thesis that coronary resistance is regulated to insure adequate blood transport for the requirements of cardiac oxygen metabolism.

Evidence of hemoconcentration was observed during the action of Hypertensin. The resulting hyperglycemia might have been a consequence of stimulation of the adrenal medulla. Under the apparent adverse conditions of increased vasoconstriction, peripheral hypoxia, greater glycoenitylosis and increased left ventricular work, the heart muscle cells were able to utilize lactate ions at a 4-fold greater rate (2.5 to 10.7 mg./100 Gm./min.). Other workers have suggested that the utilization of lactate is related to the amount available in the arterial blood.

Summary
Pharmacodynamically induced changes in left ventricular work, using an intravenous infusion of Hypertensin, were studied in intact anesthetized dogs. Coronary and systemic hemodynamics and cardiac metabolism under a combined morphine-1-Dial'-urethane-pentobarbital-sodium (MDUP) anesthesia were measured during the action of the drug. The significant hemodynamic changes induced were increased mean arterial pressure and increased left ventricular work. A greater cardiac lactate utilization during the Hypertensin infusion was the only change in cardiac metabolism. The oxygen tension in left ventricular venous blood was not reduced during the action of the drug.

Acknowledgment
We wish to thank Dr. C. Biegel for her cooperation in the supervision of analytic methods and Eleanor Sorrentino and Francis Kleckner for their technical assistance.

Summario in Interlingua
Pharmacodynamicamente inducite alterationes in le labor sinistro-ventricular (con le uso de infusiones intravenose de Hypertensina) esseva studiate in intacte anesthesiate canes. Le hemodynamic coronaria e systemic e le metabolismo cardiac sub un anesthesie combine per MDUP (= morphia, Dial, urethano, pentobarbital e natrium) esseva mesurate durante le action del droga. Le significative alterationes hemodynamic inducite esseva un augmento del tension arterial medio e un augmento del labor sinistro-ventricular. Un plus grande utilizacion cardia de lactate durante le infusion de Hypertensina esseva le sol alteration del metabolismo cardiac. Le tension de oxygeno in sanguine venose sinistro-ventricular non esseva reduce durante le action del droga.

References
1. RITZEL, W., ISELIN, B., KAPPELER, H., RINIZER, B., AND SCHWYZER, R.: Synthese eines hoch-wirksamen Hypertensin II-amids (l-Aspara-
Circulation Research, Volume VIII, November 1960
HYPERTENSIN ON CARDIAC METABOLISM


Effects of Hypertensin on Arterial Pressure, Heart Work and Cardiac Oxygen Utilization

IVAN E. FORTE, LOUIS POTGIETER, JERRY E. SCHMITTHENNER, A. JANE WILLIAM, A. JANE WILLIAM, HUNTER NEAL, SHAKASHIR TOUSSEF, ROBERT RICHARD and J. H. Hafkenschiel

Circ Res. 1960;8:1235-1241
doi: 10.1161/01.RES.8.6.1235

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1960 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:
http://circres.ahajournals.org/content/8/6/1235