Effects of an Inotropic Agent, RO 2-2985 (X-537A), on Regional Blood Flow and Myocardial Function in Chronically Instrumented Conscious Dogs and Anesthetized Dogs

By Henry G. Hartley, Robert M. Lewis, Craig J. Hartley, Dean Franklin, and Arnold Schwartz

ABSTRACT

RO 2-2985 produced a marked positive inotropic effect in unanesthetized, chronically instrumented dogs, measured as an increase in left ventricular dP/dt and an increase in maximum velocity of myocardial fiber shortening. Similar changes produced in dogs in hemorrhagic shock lasted for 2-3 hours. RO 2-2985 increased peripheral blood flow and caused marked increases in both coronary and renal blood flows. The drug altered the response of the renal vascular bed to subsequent norepinephrine administration. After administration of a single dose of RO 2-2985, norepinephrine produced sustained increases in renal blood flow, and this altered responsiveness to norepinephrine persisted for periods ranging from 1 to 3 weeks.

The antibiotic ionophore RO 2-2985 (X-537A) was first isolated in 1951 from a species of streptomycetes (1). Analysis of the structure of the RO drug has revealed the following (2, 3):

Pressman originated the concept and the possible applications of ionophorous antibiotics (4-7), and he and his colleagues have recently demonstrated the ability of RO 2-2985 (X-537A) to transport both divalent and monovalent cations (5, 6, 8) and to produce an inotropic action on the heart (8-11). Williamson et al. (12) and Levy et al. (13) have reported similar inotropic effects which they have attributed to a release of norepinephrine.

Recently, studies in this and other laboratories have revealed long-lasting hemodynamic effects of this ionophore (9-11, 14). We have found that a single intravenous injection of RO 2-2985 (1 mg/kg) in anesthetized dogs produces increases in blood pressure and cardiac output which last for periods of up to 7-9 hours. These same effects have been noted in dogs in which hypotension ("shock") has been induced by exteriorization of the intestines for periods of several hours. Surprisingly, in our study, no change in calculated total peripheral resistance was observed after the administration of this agent (14). Because of these prolonged inotropic and pressor responses, we think that the drug may prove useful in the treatment of certain clinical conditions associated with reduced blood pressure or low cardiac output, possibilities that were originally suggested by Pressman et al. (5, 6, 8, 10, 11, 15). In previous studies, we have described the general hemodynamic effects of RO 2-2985 under two conditions: in normal anesthetized dogs and in dogs with hypotension produced by prolonged exteriorization of their intestines. We have not evaluated the drug in other types of hypotension or under conditions in which acute operative interventions and anesthesia are not present. The present study was designed to (1) evaluate the effects of RO 2-2985 on myocardial function in the awake, chronically instrumented dog, (2) evaluate some regional peripheral vascular effects of the drug, and (3) study the effects of the drug during hemorrhage-induced hypotension. In particular, emphasis was placed on the effects of RO 2-2985 on renal and coronary blood flows, since the prognosis in shock states frequently is related to the degree to which these critical areas of the circulation are compromised.

Methods

A total of 22 mongrel dogs weighing 20-35 kg was used. Sixteen dogs were studied in the awake, chronically...
CONSCIOUS DOGS

Heart Rate and Systemic Arterial Blood Pressure.—RO 2-2985 produced a significant increase in both heart rate and systemic arterial blood pressure (Table 1). Heart rate was significantly increased 30 minutes after the injection of RO 2-2985 and remained increased ($P < 0.05$) for 2 hours after a single injection. Mean systemic arterial blood pressure was significantly increased 15 minutes after the drug injection, continued to increase for the first hour, and remained significantly above control levels ($P < 0.05$) for 2 hours after the drug
TABLE 1

Effects of RO 2-2985 on Regional Blood Flow in Conscious Dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control value</th>
<th>Average of maximum values after RO 2-2985</th>
<th>Percent change*</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>110 ± 7</td>
<td>128 ± 5</td>
<td>+16</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>101 ± 4</td>
<td>144 ± 7</td>
<td>+42</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Blood Flow Using All Techniques</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal blood flow (ml/min)</td>
<td>102 ± 15</td>
<td>187 ± 30</td>
<td>+83</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mesenteric blood flow (ml/min)</td>
<td>151 ± 6</td>
<td>174 ± 10</td>
<td>+15</td>
<td>NS</td>
</tr>
<tr>
<td>Iliac blood flow (ml/min)</td>
<td>99 ± 16</td>
<td>220 ± 29</td>
<td>+122</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Coronary artery blood flow (ml/min)</td>
<td>64 ± 10</td>
<td>143 ± 18</td>
<td>+123</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Resistance Values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal resistance (mm Hg/ml flow min⁻¹)</td>
<td>1.46 ± 0.31</td>
<td>1.19 ± 0.18</td>
<td>-18</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mesenteric resistance (mm Hg/ml flow min⁻¹)</td>
<td>0.62 ± 0.04</td>
<td>0.79 ± 0.11</td>
<td>+27</td>
<td>NS</td>
</tr>
<tr>
<td>Iliac resistance (mm Hg/ml flow min⁻¹)</td>
<td>1.37 ± 0.24</td>
<td>0.94 ± 0.18</td>
<td>-31</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**Blood Flow Velocities Using Doppler Techniques**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Maximum value</th>
<th>Frequency shift</th>
<th>P</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal blood flow velocity (kHz)</td>
<td>5.2 ± 0.8</td>
<td>9.7 ± 1.4</td>
<td>+86</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Mesenteric blood flow velocity (kHz)</td>
<td>5.4 ± 0.6</td>
<td>6.6 ± 0.7</td>
<td>+22</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Iliac blood flow velocity (kHz)</td>
<td>2.8 ± 0.3</td>
<td>5.9 ± 0.8</td>
<td>+110</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Coronary blood flow velocity (kHz)</td>
<td>3.7 ± 0.1</td>
<td>8.9 ± 0.9</td>
<td>+140</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

All values are means ± se. In some experiments, after the collection of data but before or at the time of calibration of the flow probes, a malfunction occurred in the probe, such as wire dislodgment, making volume calibration of the flow probe impossible but not negating the data previously obtained relative to directional shifts in blood flow velocity in response to the drug. Because of this fact the data are presented in two forms. The results for each vascular bed are presented in terms of volume flow for all dogs in which flow calibrations were possible. In addition, the results (in terms of frequency shifts which are linear with velocity) are presented for all dogs in which the Doppler flowmeter systems were used, whether calibration was possible or not.

* Note that the percent changes listed in this table are slightly different from those shown in Figure 1. This table lists the average of the maximum value in each dog following RO 2-2985 administration, regardless of the exact time at which it occurred, but Figure 1 shows the time course for each parameter.

† Determined using a paired Student’s t-test. NS = not significant.

‡ Data from different dogs.

had been administered (Fig. 1). Thus, heart rate and arterial blood pressure tended to parallel each other, and, although in a few dogs there was a prolonged augmentation (up to 7-9 hours), heart rate and blood pressure generally returned to control levels within 2 hours 15 minutes.

Myocardial Function Studies.—Following the injection of RO 2-2985, systolic segment length significantly decreased (P < 0.05) to 93 ± 1% of the control level (Fig. 2, Table 2). Note that the pen excursion is augmented in the trace in Figure 2; diastolic length is represented by the upper level of the excursion and systolic length by the lower level. For example, in Figure 2 note that the systolic segment length prior to drug administration was 12.3 mm, and after drug administration it decreased to 11.5 mm. Systolic segment length remained significantly decreased from 1 hour 45 minutes to 3 hours 15 minutes. Overall systolic movements of the segments significantly increased, and left ventricular pressure rose (Fig. 2, Table 2). The left ventricular pressure increase in the dogs in which myocardial function was studied was significant (P < 0.05) by 45 minutes, reached a maximum at 1 hour 15 minutes, and had returned to levels not significantly different from control by 2 hours 15 minutes. Left ventricular dP/dt also increased and remained at that level for 2 hours 45 minutes after the RO injection. The velocity of segment contraction showed a similar increase...
Time course of RO 2-2985 effects on systemic arterial blood pressure and regional blood flows. Values are averages for all of the dogs studied calculated at 15-minute intervals. Left circumflex coronary artery blood flow was determined in a different group of dogs than were the other measurements shown, and values were not obtained in this group after 3 hours. Solid lines = significant alterations from control values (P < 0.05, paired t-test), and broken lines = values not significantly different from control.

(Fig. 2, Table 2). Significant increases in inotropic indexes, then, persisted for 2 hours 45 minutes after a single injection of the drug, and significant alterations in myocardial dimensions (as indicated by the ultrasonic crystals) persisted for 3 hours 15 minutes (Fig. 2, Table 2).

In contrast to the effects of RO 2-2985, none of the other known cardioactive drugs, when they were given to the same dogs, was found to be exactly similar (Table 2). Norepinephrine had a much shorter (235 ± 30 seconds) duration of action and produced a bradycardia during the time when systemic arterial blood pressure was increased, whereas RO 2-2985 significantly increased heart rate (P < 0.05) in these dogs. Epinephrine also was much shorter in its duration of action (150 ± 9 seconds) and did not produce significant changes in systolic length or total shortening in the dose used. Isoproterenol (155 ± 27 seconds) and glucagon (875 ± 100 seconds) also had shorter durations of action, and both caused a significant decrease in diastolic and systolic segment lengths. Finally, digoxin, in the dose used (0.02 mg/kg), significantly increased total shortening at 45 minutes but otherwise did not significantly alter any of the parameters measured.

To ensure that the results obtained were not due to the particular anterior wall segment chosen, one dog was instrumented with three pairs of ultrasonic crystals placed in the anterior, lateral, and posterior left ventricular walls. All three segments showed directionally similar results, suggesting that the results obtained with the anterior wall segment were representative of the myocardial wall motion in other portions of the left ventricle.

Regional Blood Flow and Vascular Resistance with Emphasis on Renal and Coronary Blood Flows.—Coronary blood flow began to increase in 61 ± 45 seconds and reached a maximum level 15 minutes after the injection of RO 2-2985 (Fig. 1, Table 1). In each dog this flow increase preceded measurable changes in ventricular function (i.e., velocity of segment contraction, dP/dt, etc.) which began 247 ± 81 seconds after the injection of the drug. At 15 minutes, the flow had increased to its peak. It decreased rapidly between 15–30 minutes and then slowly continued to decrease, but it remained significantly above control values for 3 hours after one injection of the drug. The time course of the coronary blood flow changes was not similar to that of the left ventricular function parameters measured. Coronary blood flow began to rise earlier, peaked earlier (15 minutes vs. 1 hour 15 minutes), and was decreasing during the time when left ventricular pressure, dP/dt, etc. were still increasing.

Renal and iliac blood flows both significantly increased after the drug was given but exhibited different time courses (Fig. 1). Iliac blood flow was significantly increased 30 minutes (P < 0.05) after the drug was administered, reached a maximum at 1 hour 15 minutes and had returned to levels not significantly different from control by 3 hours. In contrast, renal blood flow was significantly increased 30 minutes after RO 2-2985 was injected, reached a maximum level between 2 hours 30 minutes and 4 hours, and remained significantly increased until 5 hours 15 minutes after the single injection (Fig. 3). Because of technical difficulties, flow calibrations were possible on only four of the mesenteric probes. However, shifts in flow velocity were measured in six dogs with Doppler mesenteric probes in place. In neither instance was a signifi-
EFFECTS OF RO 2-2985 IN AWAKE DOGS

![Graph showing effects of RO 2-2985 on myocardial function in a conscious, chronically instrumented dog.](image)

**FIGURE 2**

Effects of RO 2-2985 on myocardial function in a conscious, chronically instrumented dog. Measurements were made using ultrasonic transit-time segment length crystals implanted in the myocardial wall and a Konigsberg P22 pressure transducer implanted in the apex of the left ventricle. LV = left ventricular, and dL/dt = velocity of segment length motion; 1-second and 10-second time markers are at the top of the figure just below where time is listed. Systolic segment length is represented by the bottom of the pen excursion illustrated in the topmost tracing; diastolic segment length is represented by the top of the trace. Paper speeds = 0.5 mm/sec and 25 mm/sec.

In five dogs, the renal blood flow response to norepinephrine was tested prior to the administration of the RO drug, and in these five plus three additional dogs the response to norepinephrine was tested at several different times after the administration of this agent. A marked alteration in the pattern of the renal blood flow response to norepinephrine occurred after RO 2-2985 administration and persisted for periods of 1-3 weeks before gradually returning to normal (Figs. 4 and 5). Prior to the administration of RO 2-2985, the response to a single intravenous dose of 20 μg of norepinephrine was the usual decrease in renal blood flow which returned to control values in 22 ± 1 seconds. In contrast, in the same dog 8 hours after the administration of RO 2-2985 (after renal blood flow had returned to control levels), the administration of norepinephrine was followed by a transient decrease and then a marked and sustained increase (from 133 ± 28 to 192 ± 30 ml/min) in renal blood flow (Figs. 4 and 5) which lasted 25 minutes. When intravenous infusions of norepinephrine were given after the administration of RO 2-2985, renal blood flow markedly increased and continued to increase in a dose-dependent manner in excess of 300% of control despite the continuous intravenous infusion of norepinephrine. This alteration in the response to norepinephrine infusion persisted for 1-3 weeks after a single intravenous dose of the RO drug.

Since left ventricular pressure rather than central aortic pressure was measured in the dogs in which coronary blood flow was recorded, the relation between mean arterial blood pressure and blood flow in this vascular bed, i.e., coronary vascular resistance, could not be calculated directly in this group of dogs. However, it should be noted that in each dog coronary blood flow increased prior to any increase in the measured...
TABLE 2
Comparison of the Effects of RO 2-2985 with Those of Cardioactive Drugs on Myocardial Function in Conscious Dogs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Diastolic length (% change)</th>
<th>Systolic length (% change)</th>
<th>Total shortening (% increase)</th>
<th>Maximum dP/dt (% increase)</th>
<th>Maximum shortening velocity (% increase)</th>
<th>Maximum LV systolic pressure (% increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RO 2-2985 (1 mg/kg)</td>
<td>+0.9 ± 1.1</td>
<td>-6.9 ± 1.2*</td>
<td>6.8 ± 0.9*</td>
<td>58 ± 11*</td>
<td>71 ± 24*</td>
<td>44 ± 10*</td>
</tr>
<tr>
<td>Norepinephrine (20 μg)</td>
<td>+1.1 ± 0.6</td>
<td>-1.6 ± 1.3</td>
<td>2.9 ± 0.4*</td>
<td>87 ± 17*</td>
<td>29 ± 10</td>
<td>43 ± 4*</td>
</tr>
<tr>
<td>Epinephrine (20 μg)</td>
<td>+0.8 ± 0.4</td>
<td>-2.2 ± 1.2</td>
<td>1.7 ± 0.7</td>
<td>43 ± 7*</td>
<td>30 ± 8*</td>
<td>30 ± 4*</td>
</tr>
<tr>
<td>Isoproterenol (8 μg)</td>
<td>-6.7 ± 0.8*</td>
<td>-7.1 ± 1.4*</td>
<td>2.9 ± 0.8</td>
<td>93 ± 18*</td>
<td>79 ± 20*</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Glucagon (1 mg)</td>
<td>-4.5 ± 0.8*</td>
<td>-5.7 ± 1.0*</td>
<td>0.4 ± 0.7</td>
<td>52 ± 6*</td>
<td>50 ± 15</td>
<td>0 ± 3</td>
</tr>
<tr>
<td>Digoxin (0.02 mg/kg)</td>
<td>-1.0 ± 0.3</td>
<td>-1.7 ± 0.2</td>
<td>2.25 ± 0.42*</td>
<td>15 ± 5</td>
<td>30 ± 16</td>
<td>10 ± 3</td>
</tr>
</tbody>
</table>

All values are means ± SE. All drugs were administered by rapid intravenous injection. Statistics were calculated on the original data using a paired t-test. % Change in diastolic length = (diastolic length after drug administration - control diastolic length)/control diastolic length, % change in systolic length = (systolic length after drug administration - control systolic length)/control systolic length, % shortening = (diastolic length - systolic length)/diastolic length, and % increase in total shortening = % shortening after drug administration - % shortening in the control period.

* Significantly different from control (P < 0.05).

parameters of left ventricular function, and coronary blood flow between 15 and 30 minutes was decreasing while indexes of left ventricular function were increasing; both findings suggest, but not prove, a primary coronary vascular effect of RO 2-2985.

Vascular resistance was calculated at 15-minute intervals for the iliac, renal, and mesenteric vascular beds. Iliac resistance was significantly decreased 1 hour 45 minutes after the injection of the drug, after which the values were not significantly different from control levels. In contrast, renal vascular resistance showed no significant change until 2 hours 15 minutes, when it was found to be significantly decreased. The lowest level of renal vascular resistance was found at 2 hours 30 minutes, and significant decreases persisted until 4 hours 15 minutes.

**ANESTHETIZED DOGS**

**Hemorrhagic Hypotension Studies.**—The results obtained for the dogs studied during hypotension induced by acute bleeding were similar to those previously reported for anesthetized normotensive dogs.
Effects of RO 2-2985 on the renal vascular response to norepinephrine in a conscious, chronically instrumented dog. **Top:** Renal vascular response to norepinephrine before RO 2-2985 was given. **Bottom:** Response of the same dog to a second injection of norepinephrine (20 μg) when renal blood flow had returned to control levels after administration of RO 2-2985. After the initial decrease in renal blood flow, a sustained increase in flow occurred after the dog had received RO 2-2985. In this dog, the pulsed Doppler technique was used to measure renal blood flow. Paper speed = 0.5 mm/sec.

*FIGURE 4*

Renal vascular response to norepinephrine 1 day following injection of RO 2-2985. Note the sustained increase in renal blood flow after the initial vasoconstriction. In this dog, the continuous-wave Doppler technique was used to measure renal blood flow. Paper speed = 0.5 mm/sec.

*FIGURE 5*
TABLE 3

Effects of RO 2-2985 during Hemorrhagic Shock

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Control</th>
<th>Maximum after RO 2-2985</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>6</td>
<td>52 ± 1</td>
<td>109 ± 7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>6</td>
<td>160 ± 7</td>
<td>210 ± 14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Renal blood flow (ml/min)</td>
<td>6</td>
<td>98 ± 33</td>
<td>168 ± 54</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Right strain gauge (% control)</td>
<td>6</td>
<td>100</td>
<td>222 ± 27</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Right strain gauge dF/dt (% control)</td>
<td>6</td>
<td>100</td>
<td>295 ± 46</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Left strain gauge (% control)</td>
<td>6</td>
<td>100</td>
<td>177 ± 17</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Left strain gauge dF/dt (% control)</td>
<td>6</td>
<td>100</td>
<td>210 ± 24</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Renal vascular resistance (mm Hg/ml blood flow min⁻¹)</td>
<td>6</td>
<td>0.65 ± 0.11</td>
<td>0.97 ± 0.16</td>
<td>NS</td>
</tr>
</tbody>
</table>

All values are means ± se. * Determined using a paired Student’s t-test. NS = not significant.

Discussion

Pressman (5, 6, 8, 10) was the first to demonstrate the dramatic positive inotropic action of X-537A (RO 2-2985), and he and de Guzman (9-11) subsequently reported some interesting circulatory changes and suggested a possible therapeutic role for this class of compounds.

The present data demonstrate that in a conscious, chronically instrumented, normotensive dog RO 2-2985 produces a marked positive inotropic effect and causes significant increases in coronary, renal, and iliac blood flows. Similar results were observed, at least in terms of myocardial function and renal blood flow, in the anesthetized dog during experimentally induced hemorrhagic hypotension. In addition, in the conscious dog, the drug markedly altered the subsequent responsiveness of the renal vascular bed to exogenously administered norepinephrine; this alteration appeared to persist for periods of up to 3 weeks. Although no explanation for this phenomenon is readily apparent, it is not impossible that the RO drug may somehow alter the adrenergic receptor if the latter is part of the cell membrane (20).

CHANGES IN MYOCARDIAL FUNCTION

Previous studies have shown that in anesthetized, normotensive animals (8-11, 14) and in animals made hypotensive by gut exteriorization (14) RO 2-2985 produces increases in systemic arterial blood pressure, cardiac output, left and right ventricular force and their rate of development, and maximum left ventricular dP/dt. Similar results were found in the present study in dogs in which hypotension was induced by hemorrhage prior to administration of the drug. Increases in systemic arterial blood pressure and left and right ventricular force and their rate of development were recorded under conditions in which intravascular blood volume was markedly decreased. Thus, the positive inotropic action and the increase in systemic arterial blood pressure induced by RO 2-2985 do not appear to be dependent on optimal circulating blood volume.

The results in conscious dogs demonstrate similar effects on pressure and inotropic state. In particular, increases were noted in left ventricular pressure, left ventricular dP/dt, and the velocity of myocardial wall shortening. We have observed that in anesthetized animals the drug produces a consistent increase (14) in heart rate, but Pressman and de Guzman (9-11) have reported no significant change.1 In the present study, both in conscious and in anesthetized dogs, heart rate significantly increased.

1 Dr. M. W. Osborne of Hoffmann-LaRoche has also observed a significant increase in heart rate after injection of RO (personal communication).
EFFECTS OF RO 2-2985 IN AWAKE DOGS

increased. The results of the present study also demonstrate that the drug produces a significant decrease in systolic segment length, but no significant alteration in diastolic dimensions was seen. These findings suggest that the increase in those parameters reflecting inotropic state may be a result of a direct increase in left ventricular contractility rather than simply a reflection of the Starling mechanism. The comparison between RO 2-2985 and other commonly used inotropic agents indicates relative potency and differences between the RO drug and the other agents studied. The duration of action of RO 2-2985 is longer than that of a single intravenous injection of any of the other drugs studied except digoxin. Interestingly, digoxin in the dose used in the present study produced no significant effects other than an increase in myocardial wall shortening. This finding is consistent with the results of other studies on the effects of digitalis preparations on the myocardial function of both the right and the left ventricle in nonfailing healthy animals in which the normal cardiovascular control mechanisms are operative (21, 22). In those studies, ouabain was utilized (0.02 mg/kg), and, despite the more potent preparation, only minimal effects were found. Isoproterenol and glucagon, given in doses resulting in comparable responses in left ventricular dP/dt, did not significantly alter left ventricular pressure. Norepinephrine resulted in bradycardia during the time of maximal pressure elevation, but an increase in heart rate was measured after RO 2-2985 administration. Finally, epinephrine had a much shorter duration of action and, in the dose used, did not result in significant changes in systolic length or total shortening.

CHANGES IN REGIONAL BLOOD FLOW AND VASCULAR RESISTANCE WITH EMPHASIS ON THE CORONARY AND RENAL VASCULAR BEDS IN CONSCIOUS DOGS

The alterations in regional blood flow which were produced by RO 2-2985 were similar in both anesthetized (data not shown) and conscious dogs.

FIGURE 6

Hemodynamic effects of RO 2-2985 during hypotension induced by hemorrhage (anesthetized dog). Right and left ventricular force were measured by Walton-Brodie strain gauges. Renal blood flow was measured by an electromagnetic flowmeter. Paper speeds = 35 mm/sec and 0.25 mm/sec. Time in minutes is listed at the bottom of the figure.

Circulation Research, Vol. 37, August 1975
Of particular interest was the time course of the changes in the different vascular beds studied. Coronary blood flow rapidly rose to a peak and then slowly decreased to control levels during the first 3 hours of study. Iliac blood flow increased more slowly to a peak but then returned to control levels in a shorter period of time. Renal blood flow rose least rapidly and was sustained at levels above control for a significantly longer period of time. It is noteworthy that mesenteric flow did not significantly change, underscoring the specificity of action of the drug.

The changes in vascular resistance in the different beds also showed different time courses. Since left ventricular pressure and not systemic arterial blood pressure was measured in those dogs in which coronary blood flow was monitored, coronary vascular resistance could not be calculated. However, it is of interest that 3 hours after the injection of RO 2-2985 coronary blood flow remained significantly increased, but left ventricular systolic pressure had returned to control levels 2 hours 15 minutes after the injection.

In the iliac and renal vascular beds, RO 2-2985 caused a significant decrease in vascular resistance, which occurred earlier (1 hour 45 minutes) in the iliac bed than it did in the renal bed (2 hours 15 minutes). Renal vascular resistance remained decreased for a particularly long time. Significant renal vasodilation was still present 4 hours following a single RO injection. These results demonstrate that RO 2-2985 has different and complex effects on different vascular beds, both in time course and magnitude. The increase in renal blood flow produced by this drug is of very long duration, continuing after all of the other parameters measured have returned to control levels.

It is interesting to note the difference between close arterial administration of RO 2-2985 to the vascularly isolated gracilis muscle vascular bed in anesthetized dogs, which, in a recent study, we reported resulted in vasoconstriction (14), and the effects of this agent on the limb vasculature when it is given intravenously to conscious dogs. RO 2-2985 is known to release myocardial and other stores of catecholamines (12, 23, 24) and possibly other vasoactive substances (25-27) and in this study has been shown to have profound inotropic effects in the intact conscious dog. In the present study, the net effect of the drug on limb vascular resistance was directionally different (vasodilation) in the intact conscious dog compared with its effect (vasoconstriction) when it is given close-arterially to the isolated gracilis muscle of anesthetized dogs. This difference is not surprising and may reflect systemic release of an as yet unidentified vasodilator material in the intact conscious preparation.

A particularly interesting alteration in the renal vascular response to norepinephrine was noted late in this study. The sustained vasodilation produced by norepinephrine probably represented an altered responsiveness of this particular bed; it persisted for 1–3 weeks after a single injection of the RO compound. The complex effects of RO in the circulatory system and, in particular, in the renal vascular bed are not the same as those produced by dopamine (Dr. Leon Goldberg, personal communication).

In terms of mechanism, it is rather inappropriate at this time to speculate much further than we already have previously (14) by suggesting alterations in membrane permeability. Perhaps a comment on one other possibility might not be out of order. RO 2-2985 could be effecting a release of a specific prostaglandin that in turn not only may lead to a greater availability of a critical pool of calcium but also may alter the renal actions of endogenous catecholamine agonists. Both of these possibilities with regard to the prostaglandins have been enumerated (28–30) and probably are worth pursuing.

Addendum

Since this manuscript was submitted and revised, we have completed several other studies that confirm a direct effect of RO 2-2985 on the coronary vasculature, independent of the sympathetic nervous system. 6-Hydroxydopamine pretreatment (which abolishes both reflex and tyramine-induced sympathetic discharge) does not qualitatively alter any of the effects of RO 2-2985; reserpine pretreatment of both anesthetized and conscious dogs, however, abolishes the positive pressor, inotropic, and chronotropic effects of the RO drug but has no effect on the peak increase in coronary blood flow (Fed Proc 34:722, 1975). These data confirm our previous experiments (Proceedings of the International Symposium on Calcium Binding Proteins, Warsaw, 1973) and are consistent with reports of deGuzman and Pressman (11 and Circulation 49 (Suppl III): 111-36, 1974). The data also emphasize the complexities of reserpine treatment (14). In terms of the effect of RO 2-2985 on the myocardium, an anesthetized dog was treated with 6-hydroxydopamine (50 mg/kg total dose administered in divided doses for 6 days); hyperventilation, carotid artery occlusion, and tyramine administration (0.3 ml of 0.1M, five times) produced no change in arterial blood pressure, right and left ventricular force and their derivatives, and coronary and renal blood flows. Administration of RO 2-2985 produced the same positive inotropic effect and increase in coronary and renal blood flows as it did in control experiments. This finding constitutes presumptive evidence that a general release of catecholamines is not the mechanism by which
the RO drug exerts its action on the cardiovascular system.

Acknowledgment

The assistance of the following people is gratefully acknowledged: Mr. Fred Dial, Mr. Frank Dunn, Mr. W. Scott Kemper, Mr. Daniel McKown, Mr. Ronald Munson, and Mr. Fred Werner. The authors would also like to thank Dr. Ronald Kunzman of Hoffmann-LaRoche, Inc., for his generosity in supplying us with the RO drug. Special thanks are extended to Dr. Leon I. Goldberg for his assistance and advice regarding the effects of dopamine.

References

2. JOHNSON SM, HERRIN J, LIW SJ, PAUL IC: Crystal structure of a barium complex of antibiotic X-537A, BaC_{34}H_{53}O_{8}H_{2}O. Chem Commun, No. 1, pp 72-73, 1970
6. PRESSMAN BC: Carboxylic ionophores as mobile carriers for divalent anions: Role of membranes in metabolic regulation. Proceedings of a Symposium held at the University of Nebraska Medical School, Omaha, Nebraska, 1972, edited by MA Mehlmman and RW Hanson. New York, Academic Press, 1972, pp 149-164
25. NAKAGOTO Y, DOUGLAS WW: Vasopressin release from the isolated neurohypophysis induced by a calcium ionophore, X-537A. Nature (Lond) 249:479-481, 1974
27. COCHRANE DE, DOUGLAS WW: Calcium-induced extrusion of secretory granules (exocytosis) in mast cells exposed to 48/80 or the ionophores A-23187 and X-537A. Proc Natl Acad Sci USA 71:408-412, 1974
Effects of an inotropic agent, RO 2-2985 (X-537A), on regional blood flow and myocardial function in chronically instrumented conscious dogs and anesthetized dogs.

H G Hanley, R M Lewis, C J Hartley, D Franklin and A Schwartz

doi: 10.1161/01.RES.37.2.215

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1975 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/37/2/215

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/