Hemodynamic and Biochemical Effects of a New Positive Inotropic Agent

ANTIBIOTIC IONOPHORE RO 2-2985

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ABSTRACT

The antibiotic ionophores RO 2-2985 (Hoffman-LaRoche; X537A) and A23187 (Lilly) produced an abrupt release of previously bound calcium from isolated sarcoplasmic reticulum. Only RO 2-2985 produced a significant inhibition of sodium, potassium-adenosinetriphosphatase activity. In anesthetized, open-chest dogs, RO 2-2985 caused a marked increase in right and left ventricular contractile force, aortic and coronary flow, and mean central aortic blood pressure with modest changes in heart rate and no change in calculated mean peripheral resistance. A single injection of 1 mg/kg produced a sustained augmentation of mean blood pressure from a control of 45 mm Hg in dogs that were in induced shock to approximately 125 mm Hg for a period up to 7-9 hours. At that time, tyramine produced a release of catecholamines, and norepinephrine and isoproterenol produced a marked increase in force of contraction. Dogs that were treated with reserpine did not respond to the drug. RO 2-2985, but not A23187, produced positive inotropism in isolated rabbit atrial and perfused ventricular preparations. RO 2-2985 still produced an augmentation in blood pressure after β-receptor blockade. It is suggested that RO 2-2985 acts by a mechanism involving a very slow release of humoral substances, by a direct effect on a specific calcium-proton exchange, or by both. It is thought that this agent has potential therapeutic applications.

KEY WORDS heart failure sarcoplasmic reticulum shock calcium-proton exchange adenosinetriphosphatase activity dogs rabbits cats guinea pigs catecholamines reseprine contractile force aortic flow coronary flow arterial blood pressure heart rate peripheral resistance

Three crystalline antibiotics were isolated from species of Streptomyces in 1951 (1). One of these, referred to as X537A and presently coded RO 2-2985 (Hoffmann-LaRoche), has been studied in detail with respect to its biosynthesis and its structure. A variety of studies, including x-ray crystallographic analysis of the barium salt, have suggested the structure illustrated in Figure 1 (2).

Pressman et al. (3, 4) pioneered the concept of ionophoric substances that function as important mobile cation carriers. He has recently measured the divalent ion affinities of RO 2-2985 and shown that the drug is able to bind barium, strontium, calcium, and magnesium in decreasing order of affinity. The drug also binds monovalent cations. An interesting property of RO 2-2985 is its ability to form complexes with organic amines such as catecholamines (with a preference for norepinephrine over epinephrine) and to transport them (5). Pressman (6) has also shown that the drug can initiate a contraction of aortic smooth muscle, and recently he has reported that it produces an increase in the force of contraction of a dog heart without a change in the frequency of contraction. Moreover, he has indicated that reserpinized preparations respond like controls. We have reported (7, 8) that the antibiotics RO 2-2985 and A23187 produce a marked, verapamil-insensitive release of calcium from and an inhibition of calcium binding to isolated cardiac sarcoplasmic reticulum. Therefore, it appears that these substances might act as positive inotropic agents by increasing the "activator" or available intracellular calcium. In preliminary experiments, we have found that RO 2-2985 causes

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FIGURE 1

Structure of RO 2-2985.

an increase in the force of contraction of an isolated, beating rabbit atrial preparation, thus confirming the studies of Williamson et al. (9). These authors have reported that reserpinized preparations or preparations treated with β-receptor blocking agents do not respond to the drug. In pilot experiments, we have confirmed their reserpine experiments. Therefore, it seems that the ionophores could alter contraction of the heart simply by releasing catecholamines, although reserpine has a number of actions unrelated to catecholamines. Since the release of norepinephrine from adrenergic nerve endings specifically requires calcium, the action of the antibiotics could involve an alteration in calcium permeability which then leads to a slow, modulated release of transmitter. If this hypothesis is indeed correct, then these agents are potentially useful in a number of clinical situations.

Accordingly, we studied the effects of the inotropic ionophores on various hemodynamic and contractile parameters in an anesthetized dog, on a vascularly isolated canine gracilis muscle preparation perfused with blood at constant flow and on isolated, beating rabbit, guinea pig, and cat atrial and perfused rabbit ventricular preparations. We also used a dog that had been placed in shock to reduce its mean blood pressure to a low level. Both control and reserpinized animals were tested. Two possible biochemical systems involved in the control of calcium in cardiac muscle cells were studied: the sarcoplasmic reticulum and the sodium-potassium-adenosinetriphosphatase transport enzyme system.

Methods

IN VIVO CANINE STUDIES

Mongrel dogs were anesthetized with sodium pentobarbital (34-50 mg/kg, iv), and respiration was controlled with a Harvard model 606 respirator pump. Catheters were placed in the left femoral artery and vein and the left axillary artery, and a thermoprobe was placed in the right femoral vein. A left thoracotomy was done at the fourth intercostal space, and Walton-Brodie strain gauges were placed across the free walls of the left and right ventricles. A catheter for drug infusion was placed in the right atrium. Electromagnetic flow probes (Biotronex Laboratory) were placed around the ascending aorta and the proximal left circumflex coronary artery approximately 2 cm distal to its origin. Arterial blood pressure was monitored by the catheter in the axillary artery using a Statham P23Dd transducer. A Brush model 200 recorder was used for all measurements.

The following parameters were monitored: blood pressure, heart rate, aortic blood flow, left circumflex coronary artery blood flow, and left ventricular and right ventricular force as measured by the Walton-Brodie strain gauges. Derivatives from the Walton-Brodie strain gauges were also recorded; they were calculated electronically by a Biotronex Laboratory differentiator. Repeated measurements of arterial and venous PO2, PCO2, and pH were made during all experiments by a Corning 165 blood gas analyzer. The temperature, monitored by a Yellow Springs thermoprobe, remained constant at 37 ± 2°C throughout all experiments. In approximately half of the experiments, a midline abdominal incision was made, and the intestines were exteriorized for 2-4 hours prior to continuing with the experiment to lower the mean blood pressure to a shock level. No difference in results was observed between the dogs in which the intestines had been exteriorized and those in which no abdominal incision had been made. After a control period (0.25-3 hours) during which the parameters were measured, each dog received an intravenous or right atrial injection of RO 2-29851 (0.25-5.0 mg/kg). No difference occurred in the results because of the site of injection or the carrier substance used. We employed dimethylsulfoxide (DMSO) as the carrier in some experiments but later changed to a solution containing benzyl alcohol, anhydrous alcohol, and propylene glycol (BAP). The parameters measured during the control period were then followed for up to 7 hours. At this time, test doses of tyramine, epinephrine, isoproterenol, and norepinephrine were administered, and the responses to these injections were recorded. In some experiments, ouabain was given.

Generously supplied by Dr. Ronald L. Kuntzeman.
An additional seven dogs were treated with reserpine (average total dose 220 mg, im and sc injections) over a period of 7-10 days. The same experimental protocol was then followed with these dogs, and the results were recorded over the same period of time. At the end of each experiment, the electromagnetic flow probes were calibrated using gravity-flow drainage techniques.

In three dogs, the effect of intra-arterial perfusions of blood containing RO 2-2985 (2 mg/100 ml) was evaluated utilizing the vascularly isolated, denervated, reservoir-perfused gracilis muscle preparation described by Renkin and Rosell (10). The gracilis muscle was surgically isolated, the fascia surrounding it was removed, and both tendons were tightly ligated with interrupted sutures, leaving only the major artery and vein and the nerve supply intact. Blood was collected from the dog at the beginning of the experiment simultaneously into three reservoirs and was diluted 20% with Ringer's solution. RO 2-2985 was added to one reservoir in the dose level previously noted. The artery to the gracilis muscle was then cannulated with polyethylene tubing, and perfusion was begun using blood from the control reservoir delivered via an oil-displacement pump. This pump was designed so that blood flow was held constant in the absence of marked alterations in vascular resistance. The vein was then cannulated and venous outflow was directed through a drop rate meter similar to the one described by Goldschmidt and Lindgren (11). Perfusion pressure was measured using a T-tube from the inflow and a Statham P23Dd transducer. Following the onset of perfusion, 10-15 minutes was allowed to elapse while the gracilis muscle stabilized. Without interrupting blood flow, the blood perfusing the muscle was then switched to the reservoir containing RO 2-2985, and perfusion pressure and flow were recorded for approximately 10 minutes. Perfusion was then switched back to control blood, and pressure and flow in the muscle were continuously recorded. During the experiments, the gracilis muscle was kept warm (37-39°C) with a heat lamp and moistened with warm (37-39°C) with a heat lamp and moistened with.

During the experiments, the gracilis muscle was kept warm (37-39°C) with a heat lamp and moistened with Ringer's solution and heparin. RO 2-2985 was added to the aortic root via a Statham P23Dd transducer. The mannitol was unpaced and the left atrium was paced with a stimulator. Both atria were attached by a suture to a Statham force transducer (350 ohms), and tension was adjusted for maximum contractility. The drugs were added to the solution and mixing was accomplished by continuously bubbling a 95% O₂-5% CO₂ mixture through the atrial bath. The temperature of the bath was maintained at 37°C. Force and dF/dt were recorded on a Grass model 7 polygraph. The data obtained were expressed as a percentage of their value during the control state prior to drug intervention.

**BIOCHEMICAL STUDIES**

**Sodium, Potassium-Adenosine triphosphatase.**—The transport enzyme system was isolated from dog and calf hearts and brains by previously described procedures (12, 13) and from the outer medulla of canine kidneys by a procedure very recently described (14). This procedure is a significant modification of previously employed methods and yields a final preparation having a very high activity in the range of 1500 μmoles inorganic phosphate/mg protein hour⁻¹. The enzyme preparations from the medulla, the heart, and the brain contain insignificant ouabain-insensitive components. Enzyme assay and measurements of ³H-ouabain binding were carried out by procedures previously detailed (12, 15).

**Cardiac Sarcolemmal Reticulum Fragments (Cardiac Relaxing System).**—The isolation and assay procedures have been fully described in previously published reports (16, 17). Both a dual-beam spectrophotometric method and a Millipore filtration procedure were employed to study calcium binding and calcium release from isolated sarcoplasmic reticulum fragments.

The antibiotic ionophore RO 2-2985 was generally dissolved in DMSO, ethanol, or BAP. The results obtained using all three solvents were essentially the same, although ethanol appeared to be an inferior carrier in...
that effects of the drug were minimum probably due to
the inability of the drug to get to a membrane site of ac-
tion. Accordingly, BAP was selected as the best carrier,
since it appears to be the least potentially toxic at this
time (Dr. R. Kuntzman, Hoffman-LaRoche, personal
communication).

Results

PHYSIOLOGICAL EXPERIMENTS

Canine Preparations.—A single injection of RO
2-2985 into a catheter placed in the right atrium or
into the femoral vein caused a marked series of
changes beginning within 1 minute. Significant aug-
mentation of the following parameters were noted:
systemic arterial blood pressure, left ventricular
force, and right ventricular force (Fig. 2). Aortic
and coronary flow were markedly increased (Table
1). Small or no changes were noted in the elec-
trocardiogram or in vascular resistance (Table 1).
The inotropic effects of the drug usually began
within 0.5–1 minute following injection regardless
of the route of administration. The maximum
effects on blood pressure, however, were not ob-
served until an average of 49 minutes after admin-
istration. It should be noted that the particular car-
rier substance used in this experiment, DMSO, pro-
duced no effect on blood pressure and only insig-
nificant changes in the other measured parameters.
It is significant that a small dose of the drug (1 mg/
kg) produced sustained increases in all of the
parameters indicated; these increases persisted in
some cases for many hours. For example, in two
dogs in which the mean blood pressure had drop-
ped to about 45 mm Hg after the intestines had
been exteriorized for about 3–5 hours, an increase
in blood pressure up to approximately 125 mm Hg
was produced which lasted from 7–9 hours, at
which time the dog was killed.

Following the injection of RO 2-2985, systemic
arterial blood pressure, left ventricular force, right
ventricular force, and force derivatives from both
ventricles were all significantly increased (Fig. 2,
Table 1). In several experiments, tyramine (0.5 ml
of 0.1M) was injected after 7 hours of treatment
with RO 2-2985. A response no different from con-
trol was measured (50% increase in right and left
ventricular contractility).

It is notable that the average peripheral vascular
resistance did not show a significant alteration
following injection of the drug despite the pro-
found alterations in other parameters (Table 1). In
eight of ten dogs, a slight increase in vascular resis-
tance occurred, but in the remaining two dogs
vascular resistance decreased following the injec-
tion. Because alterations in calculated vascular
resistance after systemic administration of a drug
are subject to many influences in addition to the
possible direct effect of the drug on the smooth
muscles of the blood vessels, three additional dogs
were studied to determine the direct effects of the
drug on a specific vascular bed—that of the
vascularly isolated, denervated gracilis muscle per-
fused at constant flow from a blood-containing
reservoir. When the drug was perfused into the
gracilis muscle in concentrations approximating
those that the preparation would have received
during a systemic injection, vascular resistance in-
creased in each dog. The average increase was
from 27.6 to 35.1 resistance units (mm Hg/ml flow
Table 1

<table>
<thead>
<tr>
<th></th>
<th>No. dogs</th>
<th>Control</th>
<th>Maximum values after RO 2-2985</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td>17</td>
<td>104 ± 7.4</td>
<td>148 ± 9.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>16</td>
<td>150 ± 6.7</td>
<td>178 ± 7.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Aortic blood flow (liters/min)</td>
<td>11</td>
<td>1.9 ± 0.27</td>
<td>3.2 ± 0.37</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Vascular resistance (resistance units)</td>
<td>11</td>
<td>59 ± 8.5</td>
<td>62 ± 9.7</td>
<td>NS</td>
</tr>
<tr>
<td>Left circumflex flow (ml/min)</td>
<td>9</td>
<td>101 ± 43.9</td>
<td>228 ± 64.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Left ventricular force (%)</td>
<td>17</td>
<td>100</td>
<td>168 ± 12.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Right ventricular force (%)</td>
<td>17</td>
<td>100</td>
<td>189 ± 11.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Left ventricular dF/dt (%)</td>
<td>17</td>
<td>100</td>
<td>204 ± 24.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Right ventricular dF/dt (%)</td>
<td>17</td>
<td>100</td>
<td>214 ± 21.1</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

All values are means ± se. P values were determined using a paired Student's t-test. NS = not significant.

100 mg⁻¹ wet weight muscle min⁻¹) during 7 minutes of perfusion. These results are insufficient for meaningful statistical analysis, but they do suggest that the direct vascular effect of the drug may be vasoconstriction, at least in this one specific bed.

It is noteworthy that in no experiment did PO₂, PCO₂, or pH change from control after administration of the drug or during any of the measured responses.

Studies were then directed towards determining the possible mechanisms by which these sustained responses were mediated. Five dogs were reserpinized (Serpasil, Ciba) using an average total dose of 220 mg administered intramuscularly over a period of 7–10 days. Control dogs received placebo. No significant change in heart rate or mean arterial blood pressure occurred in the reserpinized dogs even after administration of very high doses of RO 2-2985.

A dose-response experiment was carried out in two dogs using the drug in a dose range from 0.25 to 5.5 mg/kg. Limitation of data precludes definitive statistical analysis at this time, but it appears that the dogs were still responding even at the highest dose, and no adverse effects were observed. A compilation of the results obtained in all of the dogs is presented in Table 1.

Isolated, Perfused Rabbit Heart.—Administration of RO 2-2985 to this preparation produced an augmentation in contractility and rate (Fig. 3). Isolated hearts obtained from reserpinized rabbits did not respond to the drug. Administration of A23187 in a wide range of doses produced no effect on any parameter studied.

To determine if the new drug was a catecholamine releaser similar to reserpine or tyramine, two rabbits were injected with RO 2-2985 (5 mg/kg, im) and two rabbits were injected with placebo twice daily for 3 days. Isolated ventricular and atrial preparations from these rabbits responded in the normal manner to norepinephrine, isoproterenol, and tyramine, and RO 2-2985. The latter produced its usual positive inotropic effect.

![Figure 3](http://circres.ahajournals.org/)

**FIGURE 3**

Effect of RO 2-2985 on control and reserpinized isolated, perfused rabbit hearts. Reserpinized rabbits received 2.5 mg/kg, im, twice daily for 2 days. Traces represent recordings of dF/dt from the left ventricle. In this figure and in Figures 4 and 5, the paper speed was 0.5 mm/sec. A: Control. B: 3 minutes after administration of 1.0 ml of 10⁻³M tyramine. C: Control after washout. D: 3 minutes after perfusion with 9 μg RO 2-2985. E: Control after washout. F: 1 minute after addition of 40 μg of norepinephrine. In this figure and in Figures 4 and 5, the dotted line represents zero dF/dt.
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Effect of RO 2-2985 on isolated right atria of control and reserpinized rabbits. Traces represent recordings of dF/dt
A: Control. B: 1 minute after addition of 3 μM RO 2-2985. C: 1 minute after another addition of 3 μM RO 2-2985. D: 1 minute after third addition of the drug. E: 1 minute after fourth addition of the drug. F: 1 minute after fifth addition of the drug (total = 15 μM). G: Control after washout. H: 1 minute after addition of 10^−4 M tyramine (final concentration). I: Control after washout. J: 1 minute after addition of 20 μg of norepinephrine. K: Control after washout. L: 5 minutes after addition of 2 x 10^−4 M ouabain (final concentration). The chamber volume in this figure and in Figure 5 = 50 ml.

Isolated Rabbit and Cat Atria.—The usual concentration of RO 2-2985 that produced a significant increase in contractility was about 1-3 μM (Fig. 4). In contrast, the ionophore A23187 in a wide range of doses produced no effect on any parameter measured. In small doses, the effects of RO 2-2985 could be washed out and repeated again. With high doses of the drug, it was occasionally difficult to wash out the effects (i.e., return to control levels), and, when the effects were removed, it was difficult to produce a second response. At that point, however, administration of either tyramine or norepinephrine produced the usual types of effects (Fig. 4). Preparations derived from reserpinized animals exhibited the usual type of supersensitive norepinephrine response and lack of significant response to tyramine (Figs. 3 and 4). These preparations did not respond to RO 2-2985 or A23187 (Figs. 3 and 4). It is of interest that, even after large doses of RO 2-2985 to normal preparations followed by washing, the responses to norepinephrine, tyramine, and ouabain were normal (Figs. 4 and 5). The prior administration of large doses of A23187, which had no effect, did not alter the response of the preparations to RO 2-2985. Repeated administration of RO 2-2985 produced a type of tachyphylaxis, but this effect could not have been due to a release of endogenous

TABLE 2
Cardiovascular Effects of RO 2-2985 following β-Receptor Blockade* in Five Dogs

<table>
<thead>
<tr>
<th></th>
<th>Control after blockade</th>
<th>Maximum values after RO 2-2985</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td>124 ± 1.97</td>
<td>218 ± 1.97</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>96.6 ± 2.44</td>
<td>103.84 ± 4.17</td>
<td>NS</td>
</tr>
<tr>
<td>Aortic blood flow (liters/min)</td>
<td>2.28 ± 0.26</td>
<td>3.10 ± 0.30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Vascular resistance (resistance units)</td>
<td>70.77 ± 8.31</td>
<td>98.60 ± 12.10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Left ventricular force (%)</td>
<td>100</td>
<td>131.80 ± 10.47</td>
<td>NS</td>
</tr>
<tr>
<td>Right ventricular force (%)</td>
<td>100</td>
<td>180 ± 3.39</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Left ventricular dF/dt (%)</td>
<td>100</td>
<td>155 ± 20.78</td>
<td>NS</td>
</tr>
<tr>
<td>Right ventricular dF/dt (%)</td>
<td>100</td>
<td>155.20 ± 8.12</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

All values are means ± SE. P values were determined using a paired Student’s t-test. NS = not significant.

*Complete β-receptor blockade was effected after a administration of a total of 80 ml of 10 mM MJ1999 (Sotalol) by slow intravenous infusion for 30 minutes and of 5 mg of propranolol. The block was tested with 4 μg of isoproterenol and 40 μg of norepinephrine.
stores of norepinephrine, since subsequent administration of tyramine produced the usual augmentation of contractility (Figs. 4 and 5). A paced left atrial preparation responded to RO 2-2985 in the same way as did an unpaced one. A paced cat atrial preparation (Fig. 5) was much more sensitive to RO 2-2985 than was a rabbit preparation and, surprisingly, was responsive after β-receptor blockade. Similar significant results with respect to β-receptor blockade were obtained in the dog preparation (Table 2).

RO 2-2985 also produced a significant increase in the maximum developed isometric tension in electrically stimulated guinea pig left atria, but the compound A23187 (4.5 μM) had no significant effect on this preparation.

**BIOCHEMICAL STUDIES**

**Sodium, Potassium-Adenosinetriphosphatase.** — RO 2-2985, in a range of concentrations, produced a significant inhibition of ouabain-sensitive sodium, potassium-adenosinetriphosphatase. Similar results were obtained with the adenosinetriphosphatase (ATPase) isolated from either dog heart muscle or kidney medulla (Table 3). A23187, in a similar range of concentrations, had little significant effect on the ATPases. It should be emphasized that these ATPases have little or no ouabain-insensitive component, but activity varies among preparations. However, we have shown that this enzyme is kinetically identical regardless of activity (13–15). In preliminary experiments, RO 2-2985 significantly inhibited 3H-ouabain binding to both heart and kidney sodium, potassium-ATPases (35% inhibition with 80 μM RO 2-2985, 64% inhibition with 150 μM RO 2-2985, and 80% inhibition with 200 μM RO 2-2985). A23187 did not significantly alter 3H-ouabain binding to sodium, potassium-ATPases.

**Sarcoplasmic Reticulum (Cardiac Relaxing System).** — Isolated sarcoplasmic reticulum fragments from cardiac muscle actively bound calcium. This process was dependent on adenosine triphosphate (ATP). In the absence of a precipitating anion such as oxalate, after calcium had been maximally bound addition of various concentrations of RO 2-2985 produced a rapid release of calcium. This process was nonsaturable (Fig. 6).

**Discussion**

Hemodynamic, physiological, and biochemical effects of RO 2-2985 and A23187 on a number of systems have been described. Since our supply of A23187 was limited, we studied the possible effects of this drug only on isolated atrial and perfused ventricular preparations. A23187 had no effect on any parameter studied in these systems. The striking effect of RO 2-2985 on all of the physiological systems studied differentiates the action of this ionophore from that of A23187. The effects of the former ionophore were particularly dramatic in a canine preparation that was in cardiovascular shock (mean blood pressure as low as 45 mm Hg). In this case, a single injection of approximately 1 mg/kg produced a remarkable increase in cardiac performance (e.g., blood pressure rose to and was sustained at approximately 125 mm Hg) that lasted 7–9 hours. At that time we terminated the experiment.
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Effect of RO 2-2985 on isolated canine cardiac sarcoplasmic reticulum. Description of isolation and assay in Methods. Top: Trace redrawn from the recorder chart of the dual-beam spectrophotometer. A TP-dependent calcium binding by the sarcoplasmic reticulum preparation is represented by a decreased slope; calcium released into the medium from the sarcoplasmic reticulum preparation is represented by an increased slope. Bottom: Reciprocal plot of drug concentration vs. velocity of calcium binding (V).

ment, but it appeared likely that the dog would have survived longer. We know of no drug or combination of drugs that can function in precisely this manner. It should be emphasized, however, that even in a freshly anesthetized dog whose blood pressure was about 100 mm Hg or higher, the same qualitative effects observed in the dog in shock were recorded. In dogs that had been reserpinized so that their cardiac muscle did not respond to tyramine but did respond to exogenous norepinephrine in a supersensitive manner, RO 2-2985 had no observable effect. This experiment suggests that a component of the mechanism of action of the ionophore might involve the release of a humoral substance or substances, but the experiment does not exclude other possible mechanisms. A number of observations reported in this paper are inconsistent with a reserpinelike or a tyraminelike catecholamine-releasing action of RO 2-2985. For example, an apparent lack of depletion of catecholamines in rabbits treated chronically with the new drug was found. Normal responses to norepinephrine, isoproterenol, tyramine, and ouabain were recorded following the administration of RO 2-2985 acutely (up to 5 mg/kg) to dogs for periods of up to 7–9 hours. These data indicate that any release of a humoral substance resulting from RO 2-2985 administration must be dissimilar to that produced by reserpine or tyramine. In addition, pharmacologically induced responses were also normal even after prolonged administration of RO 2-2985. Furthermore, complete β-receptor blockade did not abolish the spectrum of effects of RO 2-2985, indicating that β-receptor agonist action cannot be the sole determinant of RO 2-2985-induced effects. Although it is true that reserpine treatment abolished the response to RO 2-2985, it should be noted that reserpine produces a variety of effects unrelated to catecholamine release, such as alteration of mitochondrial function and structure and membrane transport, particularly associated with calcium (18–22). It is probable, therefore, that reserpine abolished the effects of RO 2-2985 by inhibiting a critical membrane site.

The fact that the reserpinized atrial and ventricular preparations behaved in a qualitatively and quantitatively similar manner to the canine preparation corroborates the importance of the effect of the drug on membrane sites associated with development of positive inotropism.

Some years ago, we suggested that certain basic proteins, particularly histones, might function in the control of cardiac contraction and relaxation by interacting with a number of enzymatic systems (23). It is possible that one of the actions of the antibiotic ionophores is to alter in some way the distribution of certain myocardial factors, one of which could be a histone. It also should be noted that small concentrations of histone produce a highly significant alteration in the inner membrane structure of mitochondria, effecting a passive release of potassium (24–26).

We have suggested that the sodium, potassium-ATPase of cardiac muscle might be the digitalis receptor (27, 28). Cardiac glycosides interacting with the sodium, potassium–ATPase presumably produce a significant conformational change which leads to an inhibition of enzyme activity and an increase in calcium influx. The latter could be due to a sodium-calcium competition (29), or the inhibition could be secondary to increased...
calcium (28). We have reported that very low concentrations of calcium appear to substitute for sodium in stimulating \(^3\)H-ouabain binding to sodium-potassium-ATPase (30). Since RO 2-2985 significantly depressed calcium, potassium-ATPase activity and inhibited \(^3\)H-ouabain binding to sodium-potassium-ATPase and since A23187 did not have much of an effect on this enzyme, it is attractive to suggest that at least one of the mechanisms of action of RO 2-2985 in producing a positive inotropic effect involves sodium-potassium-ATPase in a manner perhaps not dissimilar to that of cardiac glycosides.

It is well known that the release of transmitters from nerve endings requires calcium; the reuptake of transmitters is an active process requiring ATP, and it is inhibited by ouabain, implying the presence of an active sodium-potassium-ATPase (31). Another possibility is that a calcium-proton or a calcium-sodium or calcium-potassium exchange process modulates intracellular calcium content (32), which in turn controls the release of endogenous humoral substance(s). RO 2-2985 transports monovalent as well as divalent cations. Recently, Estrada-O has shown that RO 2-2985 induces an exchange of protons for calcium in various membrane systems (Dr. S. Estrada-O, Symposium on Calcium Binding Proteins, Warsaw, Poland, July 1973). A23187 is, however, specific for divalent cations and does not bind or transport protons or other monovalent species. Therefore, A23187 would not initiate a calcium-proton exchange; this fact could account for the lack of action of A23187 on the cardiovascular system. A membrane-associated calcium-proton exchange represents a likely site of action, in the functional sense, for RO 2-2985. We would like to suggest the following possible mechanisms which may not be mutually exclusive: (1) interaction with specific calcium-requiring sites at nerve terminals producing a slow, sustained release of some humoral substance(s), (2) interaction with the sodium-potassium-ATPase causing an increased calcium influx, and (3) interaction with the sarcoplasmic reticulum causing a calcium release. All of these processes would yield an increase in available calcium to bind to troponin, resulting in an increased force of contraction.

It is clear from the data that RO 2-2985 is not a norepinephrine-releasing agent in a manner similar to tyramine, since even extremely large amounts of the drug do not cause a demonstrable depletion of norepinephrine. Even after 7 hours of RO 2-2985 treatment, administration of a releasing agent such as tyramine produced the usual type of catecholamine response.

Our results with the reserpinized preparations are at variance with those of Pressman (6, 33) who apparently still observed an effect of the drug in animals treated with reserpine. The differences may be due to the doses of the drugs employed.

Because of the spectrum of actions described, this drug, or analogues with this type of structure, might be of potential value in the treatment of a variety of clinical conditions, including congestive heart failure and cardiogenic shock.

After completion of this manuscript, a preliminary report by Levy et al. (34) appeared containing some data that are consistent with the described inotropic action of RO 2-2985. However, these workers reported only a transient inotropic effect induced by RO 2-2985 in rabbit left atria, and we observed markedly sustained positive inotropism. Furthermore, the cardiac preparations employed by these investigators were "... not responsive to catecholamine-releasing agents such as tyramine...," after the initial inotropic response to RO 2-2985. The difference in results might be due to the high concentrations of the drug employed by Levy et al. (34). Also, dogs were not employed in their study.

Addendum

In preliminary experiments, the action of RO 2-2985 appears to be distinct from that of glucagon.

After completion of this manuscript, a paper consistent with the data came to our attention (Pressman, B.C.: Carboxylic ionophores as mobile carriers for divalent ions. In Membranes in Metabolic Regulation. New York, Academic Press, 1972, pp 149-164).

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References


EFFECTS OF RO 2-2985, A NEW INOTROPE


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