Hemodynamic Studies of a Monoamine Oxidase Inhibitor, DL-Serine-N²-Isopropylhydrazide (RO 4-1038)

Mechanism of Hypotensive Action

By M. H. Maxwell, M.D., H. C. Gonick, M.D., L. Scaduto, M.D., M. L. Pearce, M.D., and C. R. Kleeman, M.D.

It has previously been demonstrated that the monoamine oxidase inhibitor, RO 4-1038 (DL-serine-N²-isopropylhydrazide) is a clinically useful drug in the treatment of arterial hypertension.1,2 Furthermore, all the compounds that inhibit monoamine oxidase in vivo are hypotensive in man to a greater or lesser degree.3 Although the precise manner in which the disturbances of catecholamine metabolism produced by these agents result in hypotension is speculative,4 present methodology is adequate to determine their major hemodynamic effects. RO 4-1038 would appear to be an appropriate drug to study for this purpose, since it results in a greater hypotensive response than most of the other monoamine oxidase inhibitors,1,2 and it is an analogue of iproniazid (fig. 1) and thus has the typical configuration of most of the monoamine oxidase inhibitors.

Methods and Materials

Eleven male patients with consistent diastolic hypertension of varying etiology were studied. After appropriate diagnostic tests, seven subjects were considered to have essential hypertension and in four subjects the hypertension was secondary to renal disease (two chronic glomerulonephritis, one chronic pyelonephritis, and one polycystic kidney disease). Most of the patients had severe hypertension as judged by the level of blood pressure, renal changes, and radiologic or electrocardiographic evidence of left ventricular hypertrophy. Only four, however, had azotemia as manifested by serum creatinine levels greater than 1.4 mg. per cent. Previous antihypertensive medication was discontinued for at least 4 weeks prior to the initial studies. The patients were hospitalized and studies were deferred for at least 10 days to allow stabilization of blood pressure. With two exceptions regular hospital diets or diets with 6.0 Gm. of sodium chloride were prescribed; patient I.L. was continued on a 200-mg. sodium intake, which had been started 6 years earlier following a nephrotic syndrome, and patient M.F. received a 1.0-Gm. sodium diet because of prior congestive heart failure.

With patients serving as their own controls the following parameters were measured during a control period and after hypotensive effects had been achieved with drug therapy: total exchangeable sodium and sodium space as measured with Na²⁴,5 plasma volume measured with I¹³¹-labeled albumin,6 clearances of para-aminohippurate (CpAH) and inulin (Cin) by methods previously described from this laboratory,7 direct intraarterial blood pressures measured with a Statham strain-gage manometer, and cardiac output by the dye-dilution technic with use of indocyanine green and a Colson densitometer.8 Cardiac outputs were done in duplicate. The brachial artery was catheterized with a thin-wall 18-gage needle, and arterial blood was withdrawn past the cuvette densitometer with a syringe pump at 0.4 ml. per second. The volume of tubing from arm to cuvette was 0.35 ml. The dye was injected into an antecubital vein and was washed into the central circulation with a 5-ml. injection of saline. All of the duplicate outputs checked within 15 per cent and nine of the 11 checked within 10 per cent. Total peripheral resistance was calculated from the mean blood pressure, obtained by electronic integration, and cardiac output. All cardiac and renal hemodynamic studies were conducted first in the supine and then in the upright positions. The upright position during cardiac studies consisted of tilting on a tilt-table to 85 degrees for 3 minutes. In two patients (J.H. 10-13-59 and R.H. 9-17-59) the determinations were commenced before the 3-minute period of passive tilting because of postural faintness or syncope. During the renal clearance studies the upright position consisted of quiet standing at the bedside.

Drug therapy consisted of (1) RO 4-1038 in sufficient dose (10 to 40 mg. per day) to cause

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### Table 1

#### Hemodynamic and Fluid Volume Measurements before and after Drug Therapy

<table>
<thead>
<tr>
<th>Name</th>
<th>Date</th>
<th>XCl dist (Gm.)</th>
<th>Therapy</th>
<th>Whole blood volume (ml.)</th>
<th>Plasma volume (ml.)</th>
<th>Sodium space (ml.)</th>
<th>Exchangeable sodium in body (w.)</th>
<th>Mean blood pressure (mm Hg)</th>
<th>Cardiac output (Lit/min.)</th>
<th>Total peripheral resistance (dynes sec. cm.)</th>
<th>Pulse</th>
<th>Mean blood pressure (mm Hg)</th>
<th>Cardiac output (Lit/min.)</th>
<th>Total peripheral resistance (dynes sec. cm.)</th>
<th>Pulse</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.C. Polycystic kidney disease</td>
<td>9-4-59</td>
<td>6.0</td>
<td>None</td>
<td>4170</td>
<td>2778</td>
<td>13108</td>
<td>44.3</td>
<td>140</td>
<td>4.60</td>
<td>2440</td>
<td>66</td>
<td>120</td>
<td>3.48</td>
<td>2760</td>
<td>120</td>
</tr>
<tr>
<td>R.H. Essential hypertension</td>
<td>10-12-59</td>
<td>6.0</td>
<td>None</td>
<td>5535</td>
<td>3144</td>
<td>16999</td>
<td>43.1</td>
<td>210</td>
<td>4.94</td>
<td>3400</td>
<td>60</td>
<td>193</td>
<td>2.90</td>
<td>5320</td>
<td>74</td>
</tr>
<tr>
<td>R.S. Essential hypertension</td>
<td>12-21-59</td>
<td>6.0</td>
<td>None</td>
<td>6086</td>
<td>3499</td>
<td>17468</td>
<td>42.4</td>
<td>170</td>
<td>4.94</td>
<td>2750</td>
<td>69</td>
<td>190</td>
<td>4.02</td>
<td>3781</td>
<td>85</td>
</tr>
<tr>
<td>E.L. Essential hypertension</td>
<td>8-25-59</td>
<td>6.0</td>
<td>None</td>
<td>6038</td>
<td>3690</td>
<td>19022</td>
<td>40.1</td>
<td>128</td>
<td>5.09</td>
<td>2010</td>
<td>66</td>
<td>185</td>
<td>4.99</td>
<td>2970</td>
<td>89</td>
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<tr>
<td>F.B. Essential hypertension</td>
<td>10-1-59</td>
<td>6.0</td>
<td>None</td>
<td>5287</td>
<td>2735</td>
<td>17900</td>
<td>40.1</td>
<td>155</td>
<td>6.56</td>
<td>1890</td>
<td>85</td>
<td>140</td>
<td>4.43</td>
<td>2530</td>
<td>115</td>
</tr>
<tr>
<td>H.B. Essential hypertension</td>
<td>10-5-59</td>
<td>Regular</td>
<td>RO-4 5 mg. + chloro. 1.0 gm.</td>
<td>4140</td>
<td>2345</td>
<td>13819</td>
<td>36.0</td>
<td>105</td>
<td>5.16</td>
<td>1630</td>
<td>67</td>
<td>103</td>
<td>2.78</td>
<td>2960</td>
<td>90</td>
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<tr>
<td>I.L. Chronic glomerulonephritis</td>
<td>1-14-60</td>
<td>0.2</td>
<td>RO-4 20 mg.</td>
<td>5266</td>
<td>2983</td>
<td>14257</td>
<td>36.0</td>
<td>145</td>
<td>6.27</td>
<td>1850</td>
<td>61</td>
<td>118</td>
<td>4.75</td>
<td>1990</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>2-11-60</td>
<td>0.2</td>
<td>None</td>
<td>5266</td>
<td>2983</td>
<td>17574</td>
<td>45.5</td>
<td>165</td>
<td>7.41</td>
<td>1780</td>
<td>62</td>
<td>155</td>
<td>3.10</td>
<td>4000</td>
<td>78</td>
</tr>
</tbody>
</table>
Figure 1

Structural relationship between iproniazid and RO 4-1038.

Figure 2

Changes in mean blood pressure in supine and standing positions.

an unequivocal decrease in blood pressure, and (2) RO 4-1038 plus chlorothiazide 500 mg. twice daily. The order of drug therapy and control observations was varied (table 1). With rare exceptions each regimen was maintained for at least 3 weeks before determinations were made (range: 2 to 6 weeks). Renal hemodynamic data were obtained only with the initial drug regimen
Effects of RO 4-1038

The hemodynamic data are shown in table 1 and figures 2 to 4 and are summarized in table 2. The statistical evaluation of these results is presented in table 3. Tilting the control subjects to an upright position resulted in the usual homeostatic response to this maneuver: decreased cardiac output and increased peripheral resistance with little change in mean arterial pressure. The administration of RO 4-1038 resulted in a marked reduction in mean arterial pressure in both the supine and standing positions (24 and 40 per cent, respectively). This hypotensive effect was significantly greater in the standing than in the supine position (see Appendix). Cardiac output did not change significantly from control values in either the supine or standing positions. Total peripheral resistance, however, decreased significantly, 21 per cent in the supine and 45 per cent in the standing position. The additional hypotensive response in the standing position was correlated statistically with a further decrement in total peripheral resistance (correlation coefficient 0.85) (see Appendix).

Effects of RO 4-1038 Plus Chlorothiazide

Mean values of the hemodynamic studies are shown in table 2. The effects of RO 4-1038 plus chlorothiazide are compared to RO 4-1038 alone or RO 4-1038 plus chlorothiazide) in addition to the control studies.

Norepinephrine responsiveness was tested in six additional patients with essential hypertension before and after 3 weeks of therapy with 20 mg daily of RO 4-1038. The technic employed was modified from that of Aleksandrow et al. Norepinephrine was infused at a rate sufficient to raise the systolic blood pressure approximately 30 mm Hg and the diastolic blood pressure 20 mm Hg during the control periods; empirically, this varied from 3 to 12 µg per minute. After an infusion of 5 per cent dextrose in water was started, blood pressures were measured with a sphygmomanometer during a control period of 30 minutes. All patients were semisomnolent, having retired for the night. A norepinephrine infusion was then substituted and blood pressures were measured each minute for 10 minutes. In the majority of experiments a relatively constant elevation of blood pressure was manifest after 5 to 7 minutes. When blood pressure was not constant, an average value was calculated from the readings at 5, 6, and 7 minutes. Identical procedures were employed before and after drug therapy. Results are expressed as per cent increase from baseline, since the baseline blood pressure commonly decreased during drug therapy.

Results

Table 2

<table>
<thead>
<tr>
<th>Mean blood pressure (mm Hg)</th>
<th>Cardiac output (L/min.)</th>
<th>Total peripheral resistance (Dynes sec. cm.⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supine</td>
<td>Standing</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>169</td>
<td>128</td>
</tr>
<tr>
<td>RO-4</td>
<td>169 ± 29</td>
<td>128 ± 25</td>
</tr>
<tr>
<td>RO-4 + chlorothiazide</td>
<td>132</td>
<td>123</td>
</tr>
<tr>
<td>Control</td>
<td>5.75</td>
<td>5.72</td>
</tr>
<tr>
<td>RO-4</td>
<td>5.75 ± 1.18</td>
<td>5.72 ± 1.06</td>
</tr>
<tr>
<td>RO-4 + chlorothiazide</td>
<td>5.06</td>
<td>4.29</td>
</tr>
</tbody>
</table>

Table 3

Comparison of Mean Values and Standard Deviations of Hemodynamic Data During Control Period and Following RO 4-1038

<table>
<thead>
<tr>
<th>Mean blood pressure (mm Hg)</th>
<th>Cardiac output (L/min.)</th>
<th>Total peripheral resistance (Dynes sec. cm.⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>169</td>
<td>128</td>
</tr>
<tr>
<td>RO-4</td>
<td>169 ± 29</td>
<td>128 ± 25</td>
</tr>
<tr>
<td>Change</td>
<td>41</td>
<td>14%</td>
</tr>
<tr>
<td>Per cent change from control</td>
<td>24%</td>
<td>1%</td>
</tr>
<tr>
<td>P</td>
<td>&lt;.001</td>
<td>&gt;.05</td>
</tr>
</tbody>
</table>
Table 4
Comparison of Mean Values and Standard Deviations of Hemodynamic Data during Therapy with RO 4-1038 Alone and with RO 4-1038 + Chlorothiazide

<table>
<thead>
<tr>
<th></th>
<th>Mean blood pressure (mm. Hg) Supine</th>
<th>Mean blood pressure (mm. Hg) Standing</th>
</tr>
</thead>
<tbody>
<tr>
<td>RO-4</td>
<td>128 ± 25</td>
<td>103 ± 21</td>
</tr>
<tr>
<td>RO-4 + chlorothiazide</td>
<td>123 ± 22</td>
<td>100 ± 27</td>
</tr>
<tr>
<td>Change</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Per cent change</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>P</td>
<td>&gt;.05</td>
<td>&gt;.05</td>
</tr>
</tbody>
</table>

1038 alone in table 4. When compared to RO 4-1038 alone, blood pressure did not decrease further significantly in either the supine or standing position following the addition of chlorothiazide. It would appear (table 2) that chlorothiazide resulted in increased peripheral resistance and a decreased cardiac output. That these opposing effects were of approximately equal magnitude is suggested by the absence of a net change in mean blood pressure.

The fall in cardiac output could not be consistently related to changes in total exchangeable sodium, sodium space, or plasma volume (table 1).

Renal Hemodynamics

The renal clearances are shown in table 5. The control data from these hypertensive patients demonstrate the anticipated decrease in renal plasma flow and glomerular filtration rate with an increased filtration fraction. The diminution in renal function was more marked in those patients with renal disease. Upon assumption of the standing position there was the usual homeostatic decrease in renal clearances. After therapy there was no consistent change in renal hemodynamics in the supine position. In several patients (M.F., R.H., F.B.) there was a more marked reduction in renal hemodynamics in the standing position.

Norepinephrine Infusion

The results of the norepinephrine infusions are shown in figure 5. In three patients there was no change in responsiveness to norepinephrine; in three there was an increased responsiveness as manifested by an exaggerated rise in both systolic and diastolic blood pressures. In no instance was there a decreased responsiveness as has been reported with chlorothiazide.

Discussion

From the data presented the hypotensive action of the monoamine oxidase inhibitor, RO 4-1038, is attributable almost solely to a decreased peripheral resistance. If this analogue of iproniazid is representative of the other monoamine oxidase inhibitors, their hemodynamic action is unique among the clinically useful oral hypotensive drugs.

Hydralazine produces decreased peripheral resistance, increased cardiac output, and tachycardia. It also causes marked redistribution of regional blood flow with increased renal blood flow. The ganglion-blocking agents decrease blood pressure by a reduction in cardiac output without change in the total peripheral resistance. The fall in venous and...
right heart pressures\(^{17}\) and the reversal of hexamethonium hypotension by external counterpressure during immersion in water\(^{18}\) suggest a large component of venular dilatation. The ganglion-blocking agents cause a reduction in renal blood flow and glomerular filtration rate when given acutely; the renal hemodynamics tend to increase to pretreatment levels, however, with long-term use.\(^{17}\) The early hypotensive action of the thiazides is considered to result from reduced cardiac output secondary to a contracted plasma volume,\(^{19–25}\) although there are conflicting data and opinions.\(^{9, 26–31}\) The hemodynamic effects of long-term therapy with chlorothiazide is unsettled\(^{19, 24, 25, 29}\) as is the question of whether chlorothiazide decreases the glomerular filtration rate.\(^{32}\) The newer agents, guanethidine sulfate and bretylium tosylate, appear to cause a purely sympathetic blockade without side effects indicative of parasympathetic blockade. Hemodynamically, they resemble hexamethonium in that they cause a reduction in cardiac output without significant changes in peripheral resistance,\(^{33, 34}\) and the orthostatic reduction in blood pressure can be overcome by counterpressure over the lower extremities with a "g-suit.\(^{75}\) Guanethidine has been reported to decrease renal plasma flow and filtration rate.\(^{33}\)

It has been suggested that the hypotensive properties of the monoamine oxidase inhibitors may result from sympathetic ganglionic blockade.\(^{37, 38}\) This view is based on experimental studies of the effect of monoamine oxidase inhibitors on the isolated superior cervical ganglion of the cat\(^{36}\) and on ganglionic transmission in the intact animal\(^{37}\) as well as the similarity of some of the side effects of iproniazid to those commonly seen with ganglionic-blocking agents.\(^{38}\)

Although the hypotensive effect of RO 4-1038 is more marked in the upright position, unlike the ganglionic blockers there is also a significant effect in the supine position. Further evidence against the hypothesis that the monoamine oxidase inhibitors exert their hypotensive effect by ganglionic blockade is the complete dissimilarity between the hemodynamic effects of RO 4-1038 and hexamethonium. It would appear that the hypotensive effect of RO 4-1038 is attributable almost solely to impaired arteriolar vasoconstriction without significant effect on venomotor tone.

It has also been posited that the monoamine oxidase inhibitors may interfere with sympathetic responsiveness at a more distal site.
than the ganglion,\textsuperscript{3, 39-41} presumably by direct competition for receptor sites or by the accumulation of other amines that may have a similar effect. In accord with this concept is the decreased sensitivity to norepinephrine in the isolated rabbit aorta after pretreatment with iproniazid.\textsuperscript{40} The negative results of the norepinephrine infusions in the present study, however, do not support this view. The difference in the hemodynamic effects of RO 4-1038 as contrasted with bretylium tosylate\textsuperscript{34} and guanethidine sulfate,\textsuperscript{33} both of which presumably block transmission in postganglionic sympathetic nerve fibers, is further evidence against adrenergic blockade.

Whether the hypotensive action of the monoamine oxidase inhibitors is related to their monoamine oxidase-inhibiting properties is an intriguing question. In individual patients there was no correlation between increases in urinary tryptamine excretion and blood pressure effects.\textsuperscript{1, 42, 43} Zbinden et al.\textsuperscript{3} have remarked that there are only few correlations between laboratory results and clinical observations. On the other hand, the inescapable fact remains that all of the compounds that cause monoamine oxidase inhibition in vivo result in some degree of orthostatic hypotension.\textsuperscript{4, 39} That the hypotensive effect of these compounds is not due to their hydrazine structure is indicated by the recent clinical studies with a nonhydrazine monoamine oxidase inhibitor.\textsuperscript{44} Until more is known of the intermediary metabolism of the vasoactive amines as well as the changes caused by monoamine oxidase inhibition, these questions must remain unanswered.

**Summary**

Hemodynamic studies were performed on 11 patients with severe diastolic hypertension before and after the chronic administration of the monoamine oxidase inhibitor, RO 4-1038.

Although postural hypotension was more marked, the blood pressure decreased significantly in the supine position as well. The hypotensive effect was attributable almost solely to decreased total peripheral resistance and was unrelated to changes in cardiac output, blood volume, or sodium space. Renal plasma flow and glomerular filtration rate were unchanged.

Evidence is presented which suggests that the decrease in blood pressure was not caused by adrenergic blockade.

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**Table 5**

*Inulin and PAH Clearances in the Supine and Standing Positions during Control Periods and Following Drug Therapy*

<table>
<thead>
<tr>
<th>Name</th>
<th>Therapy</th>
<th>Inulin clearance (ml./min.)</th>
<th>PAH clearance (ml./min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Supine</td>
<td>Standing</td>
</tr>
<tr>
<td>J.H.</td>
<td>None</td>
<td>92</td>
<td>77</td>
</tr>
<tr>
<td>E.L.</td>
<td>RO-4</td>
<td>85</td>
<td>69</td>
</tr>
<tr>
<td>M.F.</td>
<td>RO-4 + chloro.</td>
<td>117</td>
<td>101</td>
</tr>
<tr>
<td>R.H.</td>
<td>RO-4 + chloro.</td>
<td>33</td>
<td>10</td>
</tr>
<tr>
<td>J.C.</td>
<td>None</td>
<td>108</td>
<td>114</td>
</tr>
<tr>
<td>F.B.</td>
<td>RO-4 + chloro.</td>
<td>85</td>
<td>50</td>
</tr>
<tr>
<td>J.H.</td>
<td>RO-4</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>R.S.</td>
<td>RO-4 + chloro.</td>
<td>139</td>
<td>144</td>
</tr>
<tr>
<td>A.M.</td>
<td>None</td>
<td>117</td>
<td>57</td>
</tr>
<tr>
<td>I.L.</td>
<td>RO-4</td>
<td>80</td>
<td>43</td>
</tr>
<tr>
<td>H.B.</td>
<td>RO-4 + chloro.</td>
<td>63</td>
<td>20</td>
</tr>
<tr>
<td>R.S.</td>
<td>RO-4 + chloro.</td>
<td>58</td>
<td>44</td>
</tr>
<tr>
<td>A.M.</td>
<td>None</td>
<td>49</td>
<td>40</td>
</tr>
<tr>
<td>I.L.</td>
<td>None</td>
<td>88</td>
<td>60</td>
</tr>
<tr>
<td>J.H.</td>
<td>RO-4</td>
<td>92</td>
<td>61</td>
</tr>
<tr>
<td>I.L.</td>
<td>RO-4</td>
<td>155</td>
<td>136</td>
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<tr>
<td>J.H.</td>
<td>None</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>F.B.</td>
<td>RO-4</td>
<td>41</td>
<td>38</td>
</tr>
</tbody>
</table>

*Circulation, Volume XXVI, December 1962*
If RO 4-1038 is a representative monoamine oxidase inhibitor, then its hemodynamic action is unique among the clinically useful hypotensive drugs.

Appendix

Since the control values in the supine position differed from the control values in the standing position, it was necessary to express the changes in each parameter after drug therapy as per cent change from control value in each position. In computation of the statistical results, therefore, a log transformation device was employed. For example, in comparing the mean blood pressure change following drug administration in the supine position with the change in the standing position, the natural logarithm for each observation was derived and the mean and standard deviation were then calculated for the following two series:

\[ \log \text{MBP}_c - \log \text{MBP}_a \] supine

and

\[ \log \text{MBP}_c - \log \text{MBP}_a \] standing,

where \( \text{MBP}_c \) represents control mean blood pressure and \( \text{MBP}_a \) represents mean blood pressure following RO 4-1038.

The \( p \) value derived from these results, according to the student t test, was less than 0.05 but greater than 0.01.

When total peripheral resistance (TPR) was compared in the same manner:

\[ \log \text{TPR}_c - \log \text{TPR}_a \] supine

versus

\[ \log \text{TPR}_c - \log \text{TPR}_a \] standing,

the \( p \) value was less than 0.05 and greater than 0.01. A similar derivation for cardiac output showed no significant difference in change between the supine and standing position (\( p > 0.05 \)).

Finally a computation using standard formulas expressing the correlation between changes in MBP and TPR was made by reducing the MBP change to a single value as follows:

\[ \log \text{MBP}_c - \log \text{MBP}_a \] supine

\[ \log \text{MBP}_c - \log \text{MBP}_a \] standing.

This was then related to:

\[ \log \text{TPR}_c - \log \text{TPR}_a \] supine

\[ \log \text{TPR}_c - \log \text{TPR}_a \] standing.

The correlation coefficient was 0.85 with a \( p \) value less than 0.05.

References


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MONOAMINE OXIDASE INHIBITOR


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