Antihypertensive Effect of Riboflavin Analogs in Rats with Mineralocorticoid-Induced Hypertension

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SUMMARY This study investigated whether the riboflavin analogs, 7,8-dimethyl-10-formylmethyl isoalloxazine (FMI) and 7,8-dimethyl-10-(2'-hydroxyethyl) isoalloxazine (HEI), are effective antihypertensive agents in mineralocorticoid-induced or deoxycorticosterone acetate (DOCA)-salt hypertension. These studies are based on our previous observation that aldosterone enhances the biosynthesis of renal flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) from riboflavin, and that FMI and HEI competitively inhibit conversion of riboflavin to FMN and reabsorption of Na⁺ in the kidney of adrenalectomized rats. When 1.6 mg of FMI or HEI were administered simultaneously with 3.0 mg of DOCA, the tail systolic blood pressure (SBP) of unanesthetized rats rose only to 136 ± 5 mm Hg (standard error of the mean, SEM) compared to 163 ± 5 mm Hg during DOCA therapy alone (p < 0.0005). This hypotensive effect of FMI or HEI was noted after the fourth week of treatment and persisted through the ninth week. The rats tolerated the medication well and had no signs of riboflavin deficiency.

DOCA administration alone resulted in a 24% increase in ilioptosus muscle Na⁺ concentration (p < 0.0005), and a 0.8% increase in the water content of the muscle (p < 0.025), suggesting a positive Na⁺ balance. Administration of FMI or HEI blunted the ability of DOCA to increase muscle Na⁺ concentration (p < 0.025), water content (p < 0.01). HEI treatment of the Kyoto strain of spontaneously hypertensive rats (SHR) did not lower their mean SBP. Thus it appears that the hypotensive actions of FMI or HEI are closely associated with their ability to modify the effects of mineralocorticoids on Na⁺ balance. (Hypertension 3: 75-80, 1981)

KEY WORDS • DOCA-salt hypertension • antihypertensive agents • blood pressure • sodium • riboflavin analogs

MINERALOCORTICOID hypertension induced by chronic administration of DOCA to rats in conjunction with saline-loading simulates, in some respects, the salt-dependent form of essential hypertension in humans. Accordingly, rats with mineralocorticoid hypertension have been used in some laboratories for evaluating antihypertensive agents. Abnormalities associated with salt metabolism have been observed in many forms of experimental and clinical hypertension. An increase in total body Na⁺, due in large part to an increase in intracellular Na⁺, and a fall in intracellular K⁺ have been reported during the development of DOCA hypertension. Expansion of body fluid volumes by renal retention of Na⁺ is known to raise blood pressure (BP) in susceptible individuals. It is commonly held that this is the principal factor in the pathogenesis of hypertension caused by mineralocorticoid excess, and is of variable importance in hypertension associated with renal failure, renal artery stenosis, and essential hypertension.

We have recently reported that enhanced synthesis of renal flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) may be a factor in aldosterone-mediated reabsorption of Na⁺. Simultaneous administration of the riboflavin analogs FMI and HEI diminished the conversion of riboflavin to FMN by competitively inhibiting the enzyme flavokinase (EC 2.7.1.26). Both analogs acted as natriuretic agents in the presence of aldosterone in a dose-related fashion.

In this study we have examined whether FMI and HEI are effective antihypertensive agents in rats with mineralocorticoid salt-dependent hypertension.

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Methods

Protocol

In this study, 120 male Sprague-Dawley rats weighing 150–160 g underwent right adrenonephrectomy under pentobarbital anesthesia (5 mg/100 g body weight). Following surgery all rats were fed 1% NaCl for drinking water and Purina Rat Chow (0.062 mEq Na+ and 0.282 mEq K+/g) (Ralston Purina Company, Inc., St. Louis, Missouri) ad libitum. Approximately 60 animals were used for each of the FMI and HEI studies, which were performed 5 months apart; the FMI and HEI were generously donated by The Upjohn Company, Kalamazoo, Michigan. Two or three animals were housed in plastic cages in a room with a constant temperature of 23°C and automatic light control, with dark periods from 1800 to 0600 hours. After an initial period to condition the unanesthetized rats to the tail cuff plethysmographic method, the animals were divided into four weight-matched groups.

The rats in one group received only the vehicles for injection and served as controls. The rats in the other three groups were made hypertensive by 3.0 mg of DOCA (Steraloids, Inc., Wilton, New Hampshire) suspended in 1.0 ml of sesame seed oil administered to each rat twice weekly by subcutaneous injection for 8 or 9 weeks. Two groups also received either 1.2 or 1.6 mg of riboflavin analog in 1.5 or 2.0 ml of 0.45% NaCl respectively.

Since DOCA is considered to have 4% of the Na+ retaining activity of aldosterone, then 3.0 mg of DOCA would be equivalent to 120 μg of aldosterone. We reported previously that 40 μg of FMI or HEI (20 μg/100 g body weight) effectively counteract the Na+ reabsorption induced by 3.0 μg of aldosterone (1.5 μg/100 g body weight) in adrenalectomized rats. Thus, 1.2 mg of either analog should oppose 50% of the Na+ retention, and 1.6 mg should completely block the reabsorption of Na+ by 3.0 mg of DOCA.

DOCA dissolved in sesame seed oil is absorbed slowly over several days. Previous studies (riboflavin analog U-2113, 1953; data on file, The Upjohn Company, Kalamazoo, Michigan) have demonstrated urinary excretion of the riboflavin analog over a similar period of time. Therefore, the chosen doses of FMI or HEI should be valid since the pharmacokinetics of the hormone and the analogs are similar.

Analytical Methods

Systolic Blood Pressure

The systolic blood pressure (SBP) of the unanesthetized animals was measured six times per week by a tail cuff plethysmographic method for 2 weeks (FMI study) or 4 weeks (HEI study) prior to therapy, to condition the rats. The first study (FMI) indicated that more than 2 weeks was required to condition the unanesthetized rats to the tail cuff method since their SBPs remained elevated for at least 3 weeks.

The SBP was then measured during the treatment period on the day following the injections. Six consecutive measurements were made on each unanesthetized animal at weekly intervals and the mean was used for subsequent analyses. The pressures were recorded by the same technician at the same time of day.

Sodium Intake

Weekly studies of Na+ intake were carried out in plastic rat cages. The intake of 1% NaCl was measured during four separate 24-hour periods each week, and the daily food intake of known Na+ content was measured. The mean weekly Na+ intake was then estimated using these parameters.

Serum and Tissue Electrolytes

After decapitation of the animals, blood sera and muscle Na+ and K+ determinations were made using an Instrumentation Laboratory model IL 443 flame photometer (Instrumentation Laboratory, Inc., Lexington, Massachusetts). A specimen (~1.5 g) of the left iliopsoas muscle was excised, weighed, and frozen for subsequent tissue Na+ and K+ determinations by modifications of previously developed methods. The specimens from control and treated animals were thawed and processed simultaneously. In both studies, the wet weight of each sample was measured and the specimens dried in glass crucibles at 110°C. After recording the dry weights, the samples in the FMI study were placed in a Thermodyne-type 1400 furnace and heated for two consecutive 24-hour periods at 550°C. In the HEI study, the samples were ashed in the same furnace by heating at 200°, 300°, and 400°C for 5 minutes respectively, and then at 500°C for 24 hours. The white ashes were then dissolved in concentrated nitric acid, decanted, and rinsed with dilute nitric acid and deionized water to bring the final volume of the dissolved ash and rinse to 10.0 ml. The Na+ and K+ concentrations in the iliopsoas tissue were expressed as μEq/g dry weight. The water content of the specimens was calculated by the formula (weight loss after drying/wet weight) × 100%.

All results are expressed as mean ± SEM. Statistical analysis was carried out with the Student’s t test for unpaired data; p values of < 0.05 were considered significant.

Results

Blood Pressure

In the FMI study, there were no differences in the SBPs among the four groups during the 2 weeks prior to the initiation of drug therapy. The SBP for all groups was 145 ± 4 mm Hg and remained at this level for the first 3 weeks of drug administration. With further accommodation to the technique, the SBP of the control rats fell while that of the DOCA group began to rise significantly. Due to this change in
TABLE 1. Effect of FMI Administration on the Mean Systolic BP and Mean Na⁺ Intake of DOCA-Salt Hypertensive Rats During Treatment, Weeks 5 Through 8

<table>
<thead>
<tr>
<th>Group</th>
<th>Rats (no.)</th>
<th>SBP (mm Hg)†</th>
<th>DOCA vs control (p)</th>
<th>DOCA + FMI (p)</th>
<th>Na⁺ Intake (mEq/24 hrs/rat)†</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>129 ± 4</td>
<td>&lt; 0.0005</td>
<td></td>
<td>13.63 ± 1.19</td>
<td></td>
</tr>
<tr>
<td>DOCA</td>
<td>21</td>
<td>163 ± 5</td>
<td></td>
<td></td>
<td>18.91 ± 0.80</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>DOCA + 1.2 mg FMI</td>
<td>8</td>
<td>147 ± 5</td>
<td>&lt; 0.01</td>
<td></td>
<td>18.97 ± 1.67</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>DOCA vs 1.6 mg FMI</td>
<td>8</td>
<td>136 ± 5</td>
<td>&lt; 0.0005</td>
<td></td>
<td>18.74 ± 1.26</td>
<td>&lt; 0.025</td>
</tr>
</tbody>
</table>

*Significance determined by t test for unpaired data.
†Mean ± 1 SEM for Weeks 5 through 8.

Baseline, we have excluded the FMI data from Weeks 1 through 4 and have analyzed the mean data for Weeks 5 through 8 (table 1).

The mean SBP of the rats treated with 3.0 mg of DOCA was significantly greater than that of the controls. The mean SBP of Weeks 5 through 8 in the group also given 1.6 mg of FMI was not significantly different from that of the control animals given vehicle alone. The rise in mean SBP for the group given 1.2 mg of FMI was 48% lower than in those given DOCA alone, which was virtually identical to that predicted for this group.

In the HEI study (fig. 1), the rats were normoten-sive after 4 weeks of conditioning, and there were no significant differences in their SBPs. A small drop in mean SBP occurred between Weeks 1 and 4, and a significant rise in SBP occurred during Weeks 5 through 9 of DOCA administration. In this study, both 1.2 and 1.6 mg of HEI completely suppressed the hypertensive effects of 3.0 mg of DOCA.

![Figure 1](http://hyper.ahajournals.org/Downloadedfrom)
Serum and Tissue Electrolytes and Water Content

Blood samples taken after decapitation of the animals revealed a slightly increased serum Na⁺ concentration in the DOCA-treated groups (control = 146 ± 1.5 mEq/liter; DOCA = 148 ± 1.0 mEq/liter; DOCA ± 1.2 mg FMI = 147 ± 1.2 mEq/liter; DOCA ± 1.6 mg FMI = 148 ± 1.2 mEq/liter), but the differences were not significant when compared with the controls. Serum K⁺ levels were artificially elevated in all four groups to around 8 mEq/liter due to hemolysis and tissue injury from decapitation.

DOCA administration produced a significant increase and decrease in iliopsoas muscle Na⁺ and K⁺ concentrations respectively (table 2). We attribute the absolute differences in the Na⁺ and K⁺ values between the FMI and HEI investigations to the different methods for ashing the specimens in the two studies. The variability of the method for measuring Na⁺ and K⁺ in the FMI investigation was 2.5% and 3.5% respectively, while in the HEI study it was 4.0% and 2.5% respectively. Both FMI and HEI significantly blunted the DOCA-induced changes in muscle Na⁺ concentrations.

There was also a significant increase in the water content of the muscle tissue with DOCA treatment (table 3). This increase was reduced by FMI and HEI. The variability of the method for measuring tissue water content was 0.15%.

### Sodium Intake

The 24-hour Na⁺ intake of the various groups of rats (table 1, fig. 2) was estimated on the basis of their daily volumes of saline and food consumptions. DOCA-treated animals had a 40% to 50% greater Na⁺ consumption similar to or greater than those rats treated with DOCA alone.

### Table 2. Effect of 8 Weeks of FMI and 9 Weeks of HEI Administration, Respectively, On the Na⁺ and K⁺ Contents of Iliopsoas Muscle of DOCA-Salt Hypertensive Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Na⁺</th>
<th>K⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(µEq/g dry weight)</td>
<td>(µEq/g dry weight)</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>117 ± 2</td>
</tr>
<tr>
<td>DOCA</td>
<td>21</td>
<td>149 ± 3</td>
</tr>
<tr>
<td>DOCA + 1.2 mg FMI</td>
<td>8</td>
<td>138 ± 5</td>
</tr>
<tr>
<td>DOCA + 1.6 mg FMI</td>
<td>8</td>
<td>132 ± 3</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>184 ± 7</td>
</tr>
<tr>
<td>DOCA</td>
<td>20</td>
<td>223 ± 5</td>
</tr>
<tr>
<td>DOCA + 1.2 mg FMI</td>
<td>10</td>
<td>216 ± 8</td>
</tr>
<tr>
<td>DOCA + 1.6 mg FMI</td>
<td>9</td>
<td>196 ± 11</td>
</tr>
</tbody>
</table>

Significance determined by Student's t test for unpaired data.

### Table 3. Influence of 8 Weeks of FMI and 9 Weeks of HEI Administration, Respectively, on the Water Content of the Iliopsoas Muscle of DOCA-Salt Hypertensive Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Water content (µEq/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
</tr>
<tr>
<td>DOCA</td>
<td>21</td>
</tr>
<tr>
<td>DOCA + 1.2 mg FMI</td>
<td>8</td>
</tr>
<tr>
<td>DOCA + 1.6 mg FMI</td>
<td>8</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
</tr>
<tr>
<td>DOCA</td>
<td>20</td>
</tr>
<tr>
<td>DOCA + 1.2 mg FMI</td>
<td>10</td>
</tr>
<tr>
<td>DOCA + 1.6 mg FMI</td>
<td>9</td>
</tr>
</tbody>
</table>

Significance determined by Student's t test for unpaired data.

<table>
<thead>
<tr>
<th>Group</th>
<th>Water content (µEq/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
</tr>
<tr>
<td>DOCA</td>
<td>21</td>
</tr>
<tr>
<td>DOCA + 1.2 mg FMI</td>
<td>8</td>
</tr>
<tr>
<td>DOCA + 1.6 mg FMI</td>
<td>8</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
</tr>
<tr>
<td>DOCA</td>
<td>20</td>
</tr>
<tr>
<td>DOCA + 1.2 mg FMI</td>
<td>10</td>
</tr>
<tr>
<td>DOCA + 1.6 mg FMI</td>
<td>9</td>
</tr>
</tbody>
</table>

Significance determined by Student's t test for unpaired data.

*Mean ± 1 SEM.
RIBOFLAVIN ANALOGS AS ANTIHYPERTENSIVE AGENTS/Trachewsky

A = control (15 rats).
• = 3.0 mg DOCA (20 rats).
○ = 3.0 mg DOCA + 1.2 mg HEI (10 rats).
□ = 3.0 mg DOCA + 1.6 mg HEI (9 rats).

Discussion

This investigation was conducted to establish whether FMI and HEI are effective antihypertensive agents in mineralocorticoid-induced hypertension. We have reported that FMI and HEI are effective natriuretic agents in the presence of aldosterone. The present study conclusively demonstrates that FMI and HEI are effective antihypertensive agents in the DOCA hypertensive model and blunt the DOCA-mediated increase in muscle Na+ content. Similar results have been reported by Levine and Sarkar using the mineralocorticoid antagonist potassium canrenoate. Wambach and Higgins conducted similar experiments using progesterone as an anti-mineralocorticoid. It is interesting to note (table 2) that the differential effect of the riboflavin analogs upon Na+ and K+ content is the same in skeletal muscle as that observed in the urine in our earlier studies which demonstrated that the analogs did not affect mineralocorticoid-induced alterations in the transport of K+. The physiological response to aldosterone in the adrenalectomized animal can be divided into two separable responses, namely, the antinatriuretic and the kaliuretic response, and this has been reported for the rat and the dog. These observations and our data are compatible with the view that the antinatriuretic and kaliuretic responses to mineralocorticoids can be separated.

The saline polydipsia that occurs with DOCA administration was not abated by simultaneous treatment with FMI or HEI (table 1, fig. 2). These data support the contention that the reduction of SBP by FMI and HEI is not due to a reduced fluid exchange. Previous studies of these riboflavin analogs indicated that rats given larger daily doses of 35 mg HEI/kg body weight had a decreased weight gain over a period of months, but showed no other evidence of riboflavin deficiency; observations with FMI were essentially equivalent to those with HEI (riboflavin analogs U-2113 and U-1002, 1953, data on file; The Upjohn Company, Kalamazoo, Michigan). At the end of the investigations, the rats weighed in excess of 400 g and as such received a maximum dosage of 0.4 mg of FMI or HEI/100 g body weight twice weekly. The animals in our studies tolerated the test procedures well. They did not develop changes in hair, skin, or eye characteristic of deficiencies induced by riboflavin-free diets. The mean weight of all 105 rats at the end of both studies was 468 ± 4 g, with an average rate of weight gain of 6% per week.

DOCA administration resulted in a significant 24% increase in iliopsoas muscle Na+ concentration and a significant 0.8% increase in the water content of the muscle, suggesting a positive Na+ balance. This study demonstrates that FMI and HEI markedly blunted the ability of DOCA to increase the tissue Na+ concentration, tissue water content, and SBP.
To substantiate our conclusion that the hypotensive actions of the riboflavin analogs are closely associated with their ability to modify the effects of mineralocorticoids on Na+, we investigated the influence of HEI on the SBPs of the Wistar-Kyoto (WKY) and Kyoto strain of SHR. In preliminary studies, the mean SBP of the WKY rats was unaffected by the analog administration (unpublished results). The mean SBP of the SHRs (a non-mineralocorticoid model of hypertension) was unaltered by HEI. The lack of a BP response in the non-steroid-dependent SHR rules out an effect of the riboflavin analog independent of mineralocorticoid action.

An additional possibility that has not been excluded by these investigations is a direct effect of the analogs on the contractility of vascular smooth muscle of the DOCA-salt hypertensive rats. Since HEI had no influence on the SBP of the SHR, it seems unlikely that the analogs have a direct effect on vasculature contractility. Whether the analogs are effective after the establishment of hypertension is yet to be answered.

The data suggest that riboflavin analogs may function as a novel class of antihypertensive compounds in mineralocorticoid-induced hypertension, with no apparent side effects.

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References

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