Amines in Experimental Hypertensions

By E. A. Ohler, Ph.D., and G. E. Wakerlin, Ph.D., M.D.

A sympathomimetic amine, Paredrine, was shown to be a good antihypertensive agent in experimental renal, experimental neurogenic and spontaneous hypertensions in the dog. Other closely related amines failed to possess antihypertensive activity. Paredrine did not prevent the development of experimental renal hypertension. This amine was also without chronic effect on the blood pressure of normotensive dogs.

Among the substances involved in the development of experimental renal hypertension, pressor amines, liberated by the kidney, have been suggested. If pressor amines are produced in experimental hypertension, it was felt that the administration of amines to the hypertensive dog might enhance the enzymatic destruction of renal pressor amines, either in the kidney or in the peripheral receptor organs. Paredrine hydrobromide (p-hydroxy-a-methylphenethylamine), a sympathomimetic amine, was selected as the first amine to be studied as it had been reported by Wasti1 as a good antihypertensive agent in the rat.

Materials and Methods

All of the dogs used in this study were mongrels and selected without prejudice as to sex. Experimental renal hypertension was produced by a modification of the Goldblatt technic. Experimental neurogenic hypertension was produced by Schäfer’s2 modification of the “debuffering” operation. The spontaneous hypertensive dogs were members of a group that showed sustained mean blood pressures of over 145 mm. Hg without any operative procedure. Mean blood pressures were measured by direct femoral arterial puncture using an aneroid (Tycos) manometer.

Most of the amines* used were sympathomimetic in nature although there were certain exceptions. The amines were administered daily (except Sundays) by the route indicated to the therapeutic, prophylactic and normal group of dogs. Blood pressure determinations were usually made after the acute effect of the amine had passed away. The serum of treated dogs was assayed for possible pressor neutralizing effects by the method usually employed for the determination of antirenin.3 Precipitin tests were carried out in the usual manner. Amine sensitivity patterns were determined by the intravenous injection of a given amine and the simultaneous measurement of blood pressure in the unanesthetized, trained dog. Renal function studies were carried out utilizing commonly employed techniques. Adrenal ascorbic acid determinations were made using the technic of Sayers, Sayers and Woodbury.4 Statistical analyses were made according to the methods described by Snedecor5 for paired and group comparisons.

Results

1. Therapeutic Group

Table 1 (exps. 1 to 6) shows the effect of the daily intramuscular injection of from 1 to 4 mg. per kilogram of Paredrine on the maintenance of experimental renal hypertension. The average maximal reduction of mean femoral arterial blood pressure was 26 mm. Hg with a relative decrease (percentage decrease toward normotension) of 51 per cent.

The effect of the daily subcutaneous administration of 4 mg. per kilogram of Paredrine on the maintenance of experimental renal hypertension is shown in table 1 (exps. 7 to 9). The average maximal reduction in blood pressure was 23 mm. Hg with a relative decrease of 50 per cent.

The effect of the daily subcutaneous administration of 4 mg. per kilogram of Paredrine resulted in the blood pressure changes

* Amines were furnished either by the Research Division of Smith, Kline and French Laboratories of Philadelphia or Dr. G. A. Alles of Pasadena, Calif.
in renal hypertensive dogs shown in table 1 (exp. 10 to 21). The average maximal reduc-

Figure 1 (dog 6) illustrates a typical reduction in blood pressure of a renal hyper-

tive dog during the administration of 4 mg. per kilogram of Paredrine by the three routes of administration used. It might be

Table 1.—The Effect of Administration of “Paredrine” on the Maintenance of Experimental Renal Hypertension

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Dog No.</th>
<th>Therapeutic Schedule (4 mg./Kg.)</th>
<th>Mean Femoral Arterial Blood Pressure (mm. Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Route of Adm.</td>
<td>Weeks of Therapy</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>IM*</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>IM</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>IM</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>IM</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>IM</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>IM</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>Subcut.</td>
<td>24</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>Subcut.</td>
<td>27</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>Subcut.</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>Oral</td>
<td>167</td>
</tr>
<tr>
<td>11</td>
<td>11</td>
<td>Oral</td>
<td>108</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>Oral</td>
<td>32</td>
</tr>
<tr>
<td>13</td>
<td>13</td>
<td>Oral</td>
<td>30</td>
</tr>
<tr>
<td>14</td>
<td>14</td>
<td>Oral</td>
<td>22</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>Oral</td>
<td>26</td>
</tr>
<tr>
<td>16</td>
<td>16</td>
<td>Oral</td>
<td>13</td>
</tr>
<tr>
<td>17</td>
<td>17</td>
<td>Oral</td>
<td>22</td>
</tr>
<tr>
<td>18</td>
<td>18</td>
<td>Oral</td>
<td>13</td>
</tr>
<tr>
<td>19</td>
<td>19</td>
<td>Oral</td>
<td>7</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>Oral</td>
<td>15</td>
</tr>
<tr>
<td>21</td>
<td>21</td>
<td>Oral</td>
<td>9</td>
</tr>
</tbody>
</table>

Mean Reduction in Blood Pressure of Group = 33 ± 15.9 mm. Hg. §
* 1 mg./Kg. of Paredrine.
† Spontaneous hypertensive dog.
‡ Normotensive level unknown.
§ The reduction is statistically significant since ‘p’ is less than 0.01.

FIG. 1. (Dog 6) Plot of blood pressures of a renal hypertensive dog treated with Paredrine. A, bilateral renal artery constriction; B, 4 mg./Kg. Paredrine IM daily; C, 4 mg./Kg. Paredrine subcutaneously daily; D, 4 mg./Kg. Paredrine orally daily; E, Paredrine administration stopped. Note the gradual return of blood pressure to pretreatment levels.
pointed out that renal hypertensive dogs with severe (over 180 mm. Hg) hypertensions showed greater relative and absolute reductions in blood pressure than did dogs with less severe hypertensions (table 1).

Table 2 shows the effect of 4 mg. per kilogram of Paredrine on the maintenance of experimental neurogenic and spontaneous hypertensions. All three neurogenic hypertensive dogs showed excellent reductions in blood pressure as a result of the administration of 4 mg. per kilogram of Paredrine. However, only one of the two spontaneous hypertensive dogs exhibited a reduction of blood pressure while on Paredrine therapy (table 2). The relative decrease in blood pressure of the neurogenic hypertensive dogs was 58 per cent.

Having established that Paredrine had antihypertensive activity, other closely related compounds were sought that might also be active in this respect. Particular efforts were directed toward an amine that might possess antihypertensive activity but not have acute pressor properties. Only one other chemically related amine (1-parahydroxyphenyl-2-methylaminopropane sulfate) exhibited any antihypertensive effect. The latter amine produced a modest reduction of blood pressure in one out of three renal hypertensive dogs. Fifteen other closely related amines, most of which were modifications of the β-phenylethylamine structure, failed to exhibit any antihypertensive effect in experimental renal hypertension (table 3).

2. **Prophylactic Group**

Since Paredrine was partially effective as a treatment of experimental renal hypertension, its effect on the development of renal hypertension was also explored. Eight dogs were given 4 mg. per kilogram of Paredrine orally for periods of three to six months prior to bilateral renal constriction and for varying lengths of time after renal artery constriction. The changes in blood pressure of the group during Paredrine administration and following bilateral renal artery constriction are shown in table 4. The difference in elevation of blood pressure between the Paredrine treated and control prophylactic groups is not statistically significant since "p" (Fisher "t" table) was greater than 0.05.

3. **Normal Group**

The normal group was made up of the six normotensive dogs of the prophylactic series (table 4) plus two dogs that died after bilateral renal artery constriction with the clinical and pathologic signs of malignant hypertension. Paredrine (4 mg. per kilogram orally) was administered for three months in one dog, four months in two dogs and six months in the remaining five animals. The mean arterial blood pressure of the normotensive dogs averaged...
TABLE 3.—Amines Ineffective in Treatment of Experimental Renal Hypertension (Treatment period three months)

<table>
<thead>
<tr>
<th>Amine</th>
<th>Dose</th>
<th>Route of Adm.</th>
<th>No. of Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 2-aminoisopropylane</td>
<td>4 mg./Kg.</td>
<td>Subcut.</td>
<td>2</td>
</tr>
<tr>
<td>(&quot;Tuaamine&quot;)*</td>
<td>8 mg./Kg.</td>
<td>Subcut.</td>
<td>2</td>
</tr>
<tr>
<td>2. 1-cyclohexyl-2-aminopropionate</td>
<td>4 mg./Kg.</td>
<td>Oral</td>
<td>2</td>
</tr>
<tr>
<td>3. 1-phenyl-2-aminoethane</td>
<td>4 mg./Kg.</td>
<td>Oral</td>
<td>1</td>
</tr>
<tr>
<td>(d,l-amphetamine)</td>
<td>4 mg./Kg.</td>
<td>Oral</td>
<td>2</td>
</tr>
<tr>
<td>4. 1-phenyl-2-aminoethane</td>
<td>4 mg./Kg.</td>
<td>Oral</td>
<td>2</td>
</tr>
<tr>
<td>(d-amphetamine)</td>
<td>4 mg./Kg.</td>
<td>Oral</td>
<td>2</td>
</tr>
<tr>
<td>5. 1-hydroxy-1-phenyl-2-methyl-aminopropane (ephedrine)</td>
<td>4 mg./Kg.</td>
<td>Oral</td>
<td>2</td>
</tr>
<tr>
<td>6. 1-hydroxy-1-phenyl-2-methyl-aminopropane (d-ephedrine)</td>
<td>4 mg./Kg.</td>
<td>Oral</td>
<td>2</td>
</tr>
<tr>
<td>7. 1-parahydroxy-1-phenyl-2-aminopropane (tyramine)</td>
<td>4 mg./Kg.</td>
<td>IM</td>
<td>2</td>
</tr>
<tr>
<td>8. 1-parahydroxy-1-phenyl-2-aminobutane</td>
<td>4 mg./Kg.</td>
<td>Oral</td>
<td>2</td>
</tr>
<tr>
<td>9. 1-parahydroxy-1-phenyl-3-aminobutane</td>
<td>4 mg./Kg.</td>
<td>Oral</td>
<td>2</td>
</tr>
<tr>
<td>10. 1-parahydroxy-1-phenyl-2-isopropyl aminoethane (tyramine)</td>
<td>4 mg./Kg.</td>
<td>IM</td>
<td>1</td>
</tr>
<tr>
<td>11. 1-methyl-para-di-phenacyl-2-aminobutane</td>
<td>4 mg./Kg.</td>
<td>Oral</td>
<td>2</td>
</tr>
<tr>
<td>12. 1-methyl-para-di-phenacyl-2-aminopropane</td>
<td>4 mg./Kg.</td>
<td>IM</td>
<td>2</td>
</tr>
<tr>
<td>13. 1-hydroxy-1-methyl-para-di-phenacyl-2-aminopropane (epinephrine)</td>
<td>1 mg./Kg.</td>
<td>Oral</td>
<td>1</td>
</tr>
<tr>
<td>14. 1,3-di(p-phenacyl)-2-aminoethane</td>
<td>4 mg./Kg.</td>
<td>Oral</td>
<td>2</td>
</tr>
<tr>
<td>15. 1-(methyldihydroxy-phenyl)-N, -methyl-ethylenediamine</td>
<td>4 mg./Kg.</td>
<td>Oral</td>
<td>1</td>
</tr>
</tbody>
</table>

* Generously furnished by Dr. C. E. Roach, Eli Lilly & Co.
† Kindly furnished by Dr. M. L. Tainter, Sterling-Winthrop Research Institute.
‡ Generously supplied by Dr. M. J. Schiffrin, Hoffmann-LaRoche, Inc.

111 ± 4.9 mm Hg with a range of 84 to 133 mm Hg. During the period of Paredrine administration, the mean arterial blood pressure of the normotensive dogs showed a range of 89 to 118 mm Hg with an average of 105 ± 3.2 mm Hg. Thus the mean difference in blood pressure between the control period and the treatment period was 6 mm Hg. This difference is not statistically significant as “p” (Fisher “t” table) is greater than 0.1.

TABLE 4.—The Effect of Oral Administration of “Paredrine” on the Development of Experimental Renal Hypertension

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Prophylaxis Schedule (mg./Kg.)</th>
<th>Before Renal Artery Constriction</th>
<th>During Treatment AV. &amp; S.D.</th>
<th>During Treatment AV. &amp; S.D.</th>
<th>Mean Femoral Arterial Blood Pressure (mm. Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19P</td>
<td>1 mg.</td>
<td>106 ± 6.4</td>
<td>120 ± 11.8</td>
<td>115 ± 7.4</td>
<td>142 ± 6.3</td>
</tr>
<tr>
<td>20P</td>
<td>1 mg.</td>
<td>115 ± 7.4</td>
<td>142 ± 6.3</td>
<td>116 ± 5.3</td>
<td>155 ± 13.0</td>
</tr>
<tr>
<td>21P</td>
<td>1 mg.</td>
<td>116 ± 5.3</td>
<td>155 ± 13.0</td>
<td>117 ± 5.5</td>
<td>151 ± 12.3</td>
</tr>
<tr>
<td>22P</td>
<td>1 mg.</td>
<td>117 ± 5.5</td>
<td>151 ± 12.3</td>
<td>114 ± 7.7</td>
<td>140 ± 19.5</td>
</tr>
<tr>
<td>23P</td>
<td>1 mg.</td>
<td>114 ± 7.7</td>
<td>140 ± 19.5</td>
<td>110 ± 9.3</td>
<td>133 ± 19.5</td>
</tr>
<tr>
<td>24P</td>
<td>1 mg.</td>
<td>120 ± 10.9</td>
<td>133 ± 19.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean Rise in Blood Pressure = 32 ± 12.9. (Paredrine-Treated Group)

Mean Rise in Blood Pressure = 45 ± 13.3.* (Untreated Control Group).

(Two dogs died with malignant hypertension and are not included in the table.)

* The difference of 13 mm Hg between the two groups is not statistically significant since “p” is greater than 0.05.

4. Possible Mechanisms of Action of Paredrine in Hypertension

The serum of Paredrine-treated hypertensive dogs was withdrawn during a period when the blood pressure was reduced and was assayed for possible pressor neutralizing effects. The serum of treated dogs in amounts of 5 to 25 ml. possessed no neutralizing activity to the pressor effects of the following substances: Arterenol, Paredrine, epinephrine, Tyramine, ephedrine, 2-aminopropane, renin and hypertensin.

By means of the precipitin test no anti-substance to Paredrine or to a possible Pared-
drine–plasma protein combination could be demonstrated in the serum of Paredrine-treated hypertensive dogs.

A tolerance or decreased acute pressor sensitivity to certain amines was demonstrated in dogs on chronic amine administration. A statistically significant difference in acute pressor response between control and Paredrine-treated dogs was found with the amines listed in Table 5. In each case Paredrine-treated renal hypertensive dogs evidenced a significant reduction in pressor response to the amine administered. The decreased sensitivity or tolerance of Paredrine-treated dogs to the amines listed usually developed after roughly two weeks of Paredrine administration. Figure 2 illustrates the acute response of a dog to Paredrine before treatment and during a period of decreased sensitivity. However, a normal sensitivity pattern was displayed by Paredrine-treated hypertensive dogs to many other pressor agents, including renin, hypertensin and epinephrine. Normal dogs treated with Paredrine (members of the prophylactic group) showed the same pattern of amine sensitivity as renal hypertensive dogs. The phenomenon of tolerance or decreased sensitivity to pressor amines was also observed with other amines administered chronically as possible antihypertensive agents. Since amines other than Paredrine had little or no antihypertensive activity and yet brought about certain decreased sensitivities, and since tolerance and the antihypertensive effect did not necessarily occur together, tolerance is probably not related to the antihypertensive effects of Paredrine.

Olsen and associates reported that renal hypertensive rats were more sensitive to the pressor action of arterenol than normal rats. We were unable to confirm this finding in renal hypertensive dogs. The difference in response between a group of nine normotensive dogs (mean rise 56 ± 10.9 mm. Hg) and nine renal hypertensive dogs (mean rise 59 ± 9.6 mm. Hg) was only 3 mm. Hg and this was not statistically significant ("p" greater than 0.5). Renal function studies were carried out in four renal hypertensive dogs before and during treatment with Paredrine. The results are based on at least six clearance periods before treatment in each renal hypertensive dog and...
at least six clearance periods during the administration of Paredrine. The average renal plasma flow as determined by the clearance of para-aminohippurate during the control period was 206.8 cc. per minute per square meter and 226.2 cc. per minute per square meter during Paredrine treatment while the blood pressure level was reduced. The average glomerular filtration rate as determined by the clearance of creatinine was 64.2 cc. per minute per square meter during the control period and 74.1 cc. per minute per square meter during the administration of Paredrine. These differences are not of sufficient magnitude to be significant.

The effect of Paredrine administration on the adrenal ascorbic acid content of the male rat was determined since there is some evidence that one or more courses of injection of crude anterior pituitary extracts may also be effective as antihypertensive agents. The intravenous injection of 1.6 mg. of Paredrine into intact male rats (eight assays) caused an adrenal ascorbic acid depletion of 205 mg. per 100 Gm. This represents approximately maximal depletion and was statistically significant from a group of saline controls (12 assays) in that “p” (Fisher “t” table) was less than 0.01. Such depletion was not found in hypophysectomized rats.

**DISCUSSION**

Perhaps the most widely used treatments of experimental renal hypertension have involved the use of tissue extracts, particularly kidney. Recently Wakerlin and associates have reported consistently good antihypertensive activity in kidney extracts made only from renal cortex. Many chemically known compounds have been used as possible antihypertensive agents. Chemically, the closest compounds to Paredrine that have been reported as having antihypertensive activity are two epinephrine derivatives, 2-iodoadrenochrome and 2-bromoadrenochrome. Paredrine constitutes the first chemically known substance that persistently lowers the blood pressure of renal hypertensive dogs.

The mechanism of action of Paredrine in reducing the blood pressure of renal hypertensive dogs remains obscure. However, one might postulate that Paredrine acts by occupying the peripheral receptor organs, thus rendering less effective a pressor agent liberated by the kidney. That is, the humoral agent produced by the kidney, whatever it may be, may have a chemical structure close enough to Paredrine that both might occupy common receptors.

Attempts to treat experimental neurogenic hypertension have been largely surgical in nature. Previous successful medical treatments of neurogenic hypertension, exclusive of ganglionic and adrenergic blocking agents, have included the use of xyloquinone and quinine and quinidine. The administration of Paredrine produced striking reductions in blood pressure in the three neurogenic dogs treated.

One of two spontaneous hypertensive dogs showed an excellent reduction in blood pressure during the administration of Paredrine. Previous successful treatment of spontaneous hypertension has involved the use of renal extracts.

It is of considerable interest that Paredrine had some therapeutic effect in the three types of canine hypertension. It may be that neurogenic, renal and spontaneous hypertension have more in common than an elevation of blood pressure. Experimental and spontaneous hypertensions may involve the nervous system, endocrine glands and kidney to varying degrees.

The fact that Paredrine did not prevent the development of experimental renal hypertension nor effectively treat the early months of hypertension suggests that early and late renal hypertensions may have different pathogenetic bases. Apparently Paredrine does not produce its effect by increasing renal blood flow in the renal hypertensive dogs.

The results of the adrenal ascorbic acid assays following the administration of Paredrine suggest that Paredrine may act through a modification of existing endocrine relationships. The exact mechanism of such an action is not discernible at present.
SUMMARY

1. A sympathomimetic amine, Paredrine hydrobromide, was shown to be a good anti-hypertensive agent in 21 experiments involving 14 renal hypertensive dogs.

2. Paredrine was effective in the treatment of three neurogenic hypertensive dogs and in one out of two spontaneous hypertensive dogs.

3. Only 1 out of 15 other closely related amines evidenced any antihypertensive activity.

4. The administration of Paredrine did not prevent the development of experimental renal hypertension.

5. Paredrine was administered to eight normotensive dogs without significantly altering blood pressure levels.

6. The administration of Paredrine and other amines resulted in a decreased sensitivity to the acute pressor action of certain amines in normotensive and hypertensive dogs. Changes in amine sensitivity apparently did not reflect antihypertensive activity.

7. Paredrine administration did not significantly alter renal plasma flow or glomerular filtration rate in renal hypertensive dogs.

8. Paredrine caused maximal depletion of adrenal ascorbic acid in the intact rat but was without effect in the hypophysectomized rat.

9. No difference could be detected in the pressor response to the injection of arternol in normotensive and renal hypertensive dogs.

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REFERENCES


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