Studies with the Ganglionic Blocking Agent, Chlorisondamine Chloride in Unanesthetized and Anesthetized Dogs

By R. A. Maxwell, Ph.D., A. J. Plummer, M.D., and M. W. Osborne

The normotensive unanesthetized dog receiving chlorisondamine chloride, pentapyrrolidinium, or hexamethonium exhibits a drop in systolic blood pressure, narrowing of pulse pressure, tachycardia and relaxation of nictitating membranes. The relative potencies of these compounds are in the ratio 8:3:1 respectively. While these compounds are apparently equally well absorbed, the activity of chlorisondamine chloride is markedly prolonged. This prolonged activity of chlorisondamine chloride after oral administration is probably due, in large part, to its persistence in the tissues. Chlorisondamine chloride and hexamethonium can suppress the pressor response induced in neurogenic hypertensive dogs by application of annoying stimuli.

Since the introduction of ganglionic blocking agents in the treatment of hypertension much work has been done to develop ganglionic blocking compounds having increased potency coupled with long duration of action. As a result of this effort, tetraethylammonium has been followed by such compounds as hexamethonium, azamethonium, and, more recently, pentapyrrolidinium. These symmetrical type compounds are generally considered to block each division of the autonomic nervous system equally.

More recently we have reported on the pharmacological properties of a new, highly active ganglioplegic agent, chlorisondamine chloride. As pointed out, the structure of this compound differs from the previous methonium type compounds by the possession of an asymmetric configuration at either end of the methylene chain, by halogenation of the aromatic heterocyclic substituent, and by a shortening of the methylene chain between the quaternary nitrogens. Pharmacologically, these changes have resulted in markedly increased potency and duration of autonomic ganglionic blockade.

The present communication deals with the effects of the administration of chlorisondamine chloride to unanesthetized dogs in both acute and chronic experiments, and with the possibilities of differential blockade in various parts of the autonomic nervous system.

**Methods**

Eleven unanesthetized dogs of both sexes were employed in this study, together with 10 dogs anesthetized with 30 mg./Kg. of pentobarbital intraperitoneally. The unanesthetized dogs were selected for docility and were trained to lie in the supine position and to accept calmly femoral arterial puncture with a 22 gauge needle. On the day of an experiment food was withheld.

Drugs were administered intravenously via the cephalic vein of the foreleg and orally in gelatin capsules.

Blood pressures were recorded with the Sunborn electromanometer and polyviso recorder. The apparatus employed for puncture is our modification of a mean-pressure recording instrument originally devised by Dameshek and Loman for human studies. Clotting was prevented by a very slow, non-interfering back drip from a saline bottle. Carotid pressure of the anesthetized dogs was recorded with an Anderson glass membrane manometer.

The method of Chen and associates was employed to determine relative activity of acutely administered chlorisondamine chloride upon sympathetic and parasympathetic ganglia.

**Results**

Effect of chlorisondamine chloride on the arterial blood pressure of unanesthetized dogs. In 3 animals tested, 2 mg./Kg. orally produced, within two hours, a 25 to 40 mm. Hg depression.
of systolic pressure which lasted from 5+ to 24+ hours. No depression of diastolic pressure occurred, while heart rates increased by 80 to 90 beats per minute. As previously reported by us, this oral dose generally produced maximal relaxation of the nictitating membrane in dogs.

Intravenous administration of 1 mg./Kg. of chlorisondamine chloride to 3 dogs resulted in responses similar to those seen after oral administration, but, as would be expected, the onset was more rapid, occurring within one minute. At 24 hours systolic pressure was still reduced 15 to 30 mm. Hg, but nictitating membranes had returned to normal. At 29 hours blood pressure recovery was complete.

Comparative activity of chlorisondamine chloride, pentapyrrolidinium and hexamethonium on the nictitating membrane and arterial blood pressure. We have noticed considerable variation among animals in the dose of ganglionic blocking agent required to produce comparable relaxation of the nictitating membranes. Preparatory to a critical comparison of the hypotensive action of ganglionic blocking agents in the unanesthetized dog, it was decided to establish precisely in 3 dogs the oral doses of these drugs which were just sufficient to give maximal relaxation of the membrane. The complete dose-response relationship from slight to full relaxation was determined for each compound being studied.

Table 1A is a summary of the minimum doses required in these three animals for complete relaxation following oral administration of each blocking agent. As can be seen, on either a microgram or molar basis, in each of the three animals, potency and duration of action were in the following order: chlorisondamine chloride > pentapyrrolidinium > hexamethonium.

Dog no. 9 was selected for blood pressure studies because of adaptability to training for the procedure of femoral puncture. This dog was given the previously established equi-relaxant dose of each drug. Four mg./Kg. of pentapyrrolidinium and 15 mg./Kg. of hexamethonium caused their greatest depression of systolic peaks after two hours, followed by gradual recovery to levels which approached control at the end of six hours. These compounds did not lower diastolic pressures but did elevate heart rates by 55 to 80 beats per minute. Systolic pressures the next morning were at control levels. Chlorisondamine chloride, however, in a dose of 1.5 mg./Kg., permitted only slight recovery at the end of 24 hours. Recovery was not complete until 29 hours after drug administration.

Intravenous activity. The same 3 animals in which the minimum oral doses for total nicti-

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Chlorisondamine Chloride</th>
<th>Pentapyrrolidinium</th>
<th>Hexamethonium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose µg/Kg.</td>
<td>Relative Molar Dose/Kg.</td>
<td>Duration Hours</td>
</tr>
<tr>
<td>A. Oral administration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2000</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>1000</td>
<td>1</td>
<td>4.5</td>
</tr>
<tr>
<td>9</td>
<td>1500</td>
<td>1</td>
<td>3.75</td>
</tr>
<tr>
<td>B. Intravenous Administration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>75</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>200</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

* Doses which produced maximal relaxation in three successive tests. Reduction of these doses 15 per cent consistently produced submaximal relaxation.
† Relative molar doses/Kg. chlorisondamine chloride taken as standard.
tating membrane relaxation had been established were studied to determine the complete intravenous dose-response relationship. Table 1B is a summary of the minimal doses required in each animal for total relaxation. The order of intravenous potency and duration on both a microgram and molar basis was the same as that following oral administration (see table 1A), i.e., chlorisondamine chloride > pentapyrroldinium > hexamethonium.

Since the doses in both parts of table 1 represent minimum doses required to give total relaxation, it follows that the blood or tissue level reached after oral administration (Table 1A) must approximate the level reached following the administration intravenously (table 1B). When the ratio oral dose (μg.) i.v. dose (μg.)

for total relaxation with each drug are compared, it is observed that there is no marked or consistent trend. The ratios, in the order: chlorisondamine chloride, pentapyrroldinium, hexamethonium; are, for dog no. 7: 20, 8, 16; for dog no. 8: 13, 12, 15; and for dog no. 9: 7, 7, 15. Thus, under the conditions of this experiment, there seems to be no evidence for a distinct difference in absorption of any one of these diquaternary compounds. The more prolonged activity of chlorisondamine chloride after oral administration must, therefore, be due not to differences in absorption, but to persistence of that fraction of chlorisondamine chloride which is absorbed.

Antihypertensive effect of hexamethonium and chlorisondamine chloride. Three dogs which had been subjected to bilateral carotid sinus resection and excision of the aortic depressor nerves were used in this study. Marked pressor responses are easily induced in these animals by applying annoying stimulation. Rapid motion of the head for approximately 10 to 20 seconds will suffice to promptly elevate both systolic and diastolic arterial pressure by 150 to 200 mm. Hg with peak pressures reaching levels between 300 and 375 mm. Hg (fig. 1).

Hexamethonium and chlorisondamine chloride were tested for their ability to antagonize these pressor responses. Following administration of drugs, stimulation periods were always extended to twice the control length if the pressor responses were not forthcoming. 1.0 mg./Kg. of hexamethonium was effective in markedly suppressing pressor responses in all 3 dogs within fifteen minutes; however, by two hours the pressor response had returned to control proportions. Three hundred μg./Kg. of chlorisondamine chloride was also effective in markedly reducing the pressor response in all 3 dogs at the end of fifteen minutes. The response was more protracted than after hexamethonium and marked antagonism was still in evidence in all 3 animals at the end of six hours. By 24 hours the pressor response had returned to control dimensions (fig. 1).

Chronic administration of chlorisondamine chloride to the unanesthetized dog. A series of 8 dogs was given chlorisondamine chloride daily over a period of several months and observations were made on the femoral arterial blood pressure, heart rate, nictitating membrane relaxation and pupillary reactions.

After a marked response of each dog to a single dose (2 mg./Kg.) of chlorisondamine chloride had been demonstrated, the dogs were placed on four chronic dose schedules:

0.7 mg./Kg. three times per day: 2 dogs received this regimen. Dog no. 1 showed no activity and did not respond to a subsequent dose of 2 mg./Kg. Six mg./Kg. were required to produce full activity, except that no tachycardia occurred. Dog no. 2 exhibited moderate activity at this dose and after one month's chronic study, exhibited full responsiveness to a subsequent 2 mg./Kg. dose, except for absence of tachycardia.

1.0 mg./Kg. two times per day: this dog exhibited full activity, except for the loss of the tachycardia response, after one month of drug administration.
TABLE 2.—Doses of chlorisondamine chloride which suppress by 50 per cent (SD\textsubscript{50}) the urinary bladder contractions and blood pressure rises induced by ganglionic stimulation with DMPP.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>SD\textsubscript{50} Bladder mg./Kg.</th>
<th>SD\textsubscript{50} Blood Pressure mg./Kg.</th>
<th>Bladder SD\textsubscript{50} Blood Pressure SD\textsubscript{50}</th>
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<tbody>
<tr>
<td>1</td>
<td>55</td>
<td>95</td>
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</tr>
<tr>
<td>2</td>
<td>90</td>
<td>125</td>
<td>0.7</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>75</td>
<td>0.9</td>
</tr>
<tr>
<td>4</td>
<td>150</td>
<td>150</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>50</td>
<td>1.0</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>100</td>
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</tr>
<tr>
<td>7</td>
<td>170</td>
<td>140</td>
<td>1.2</td>
</tr>
<tr>
<td>8</td>
<td>170</td>
<td>70</td>
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</tr>
<tr>
<td>9</td>
<td>235</td>
<td>85</td>
<td>2.8</td>
</tr>
<tr>
<td>10</td>
<td>74</td>
<td>24</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Mean ± S. E. 116 ± 19 91 ± 13

20 mg./Kg., once a day: resulted in constant full activity with no loss of tachycardia response.

20 mg./Kg., once a day: 2 of 4 dogs on this dose exhibited heart rates of 124 to 164 in the mornings which slowed to 100 to 108 in the afternoon. Two dogs showed heart rates of 92 to 112 during the day.

Relative effectiveness of chlorisondamine chloride as an antagonist to contraction of the urinary bladder and pressor effect induced by 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) in anesthetized dogs. The doses suppressing the blood pressure and bladder responses by 50 per cent (SD\textsubscript{50}) for 10 animals are given in table 2. While there is considerable variation among animals in the absolute dose/Kg. required for SD\textsubscript{50}, the ratios of

\[
\frac{\text{Bladder SD}_{50}}{\text{Blood Pressure SD}_{50}}
\]

for 70 per cent (7 out of 10) of the animals fall between 0.6 and 1.2, with the ratio for the other 3 lying between 2.4 and 3.1. For the group of animals there is no statistically significant difference between the doses required for blockade of equal degree at bladder ganglia and at sympathetic ganglia.

DISCUSSION

In normotensive unanesthetized dogs, acute administration of doses of ganglionic blocking agents which give substantial systolic and diastolic drops in anesthetized dogs, produce systolic reduction, marked tachycardia but little or no decrease in diastolic pressure. Apparently cardiovascular compensatory mechanisms are markedly dampened by barbiturates.

Crumpton and associates\textsuperscript{4} suggest that reduced cardiac output is the cause of the blood pressure reduction caused by hexamethonium, but could not comment on whether this reduction was secondary to decreased venous return. Since we\textsuperscript{5} were unable to demonstrate any direct effect of chlorisondamine chloride on the isolated heart of the cat or dog, we favored the latter possibility. Chlorisondamine chloride can block the depressor response invoked by peripheral vagal stimulation, and subsequent deblocking of the ganglia with neostigmine is then followed by the normal depressor response to peripheral vagal stimulation,\textsuperscript{10} therefore it is not improbable that the tachycardia following chlorisondamine chloride is due to the reduction of the normally high vagal tone governing the heart rate of the dog.

Chronic administration of chlorisondamine chloride can result in: (1) with low dosage, a state of insensitivity so that a larger than normal quantity of this compound is then required to elicit responses; (2) a continued responsiveness with optimum amounts of the drug, with the exception that the tachycardia response is generally lost. Although the results obtained with the acute test of Chen and associates,\textsuperscript{8} show no statistically significant difference between sympathetic and parasympathetic blocking effects of chlorisondamine chloride, it is not inconceivable that the loss of the tachycardia response during chronic administration of this compound is a reflection of a return of vagal control to the heart. It is also clear that intravenous doses of 1.0 mg./Kg. of chlorisondamine chloride produced more prolonged effects on the blood pressure than on the nictitating membrane. Furthermore, Plummer and associates\textsuperscript{11} report that doses of chlorisondamine chloride producing marked hypotension in the dog, increase rather than decrease the motility of the descending colon, and Crimson\textsuperscript{10, 12} and...
EFFECTS OF CHLORISONDAMINE CHLORIDE

Winsor report that constipation, which is commonly seen in clinical ganglionic blockade, is not a difficult problem in chlorisondamine chloride treated patients.

The writers feel that the evidence presented here, though inconclusive, when coupled with data of other authors, suggests that a varied responsiveness of ganglia to chlorisondamine chloride may exist.

SUMMARY

The trained, normotensive unanesthetized dog responds to administration of ganglionic blocking agents by exhibiting a drop in systolic pressure, narrowing of pulse pressure, marked tachycardia and relaxation of nictitating membranes.

Chlorisondamine chloride can suppress the pressor responses induced in neurogenic hypertensive dogs by application of annoying stimuli.

A study of the minimal doses required for total relaxation of the nictitating membrane following oral and intravenous administration of chlorisondamine chloride, indicate that its more prolonged oral activity as compared to hexamethonium and pentapyrrolidinium, is most likely due to its persistence in the tissues and is not dependent upon its being better absorbed than these compounds.

Administration of equipotent oral doses of chlorisondamine chloride, pentapyrrolidinium and hexamethonium to the same unanesthetized dog resulted in comparable systolic pressure falls lasting approximately 24, 6 and 6 hours respectively.

Chronic administration of chlorisondamine chloride to dogs can result in (1) relative insensitivity of the animal to the drug with subeffective dosage and (2) constant responsiveness except for loss of tachycardia with fully active doses.

There was no statistically significant difference in a group of 10 animals given chlorisondamine chloride intravenously between the mean doses required to depress by one-half the blood pressure responses and urinary bladder contractions induced by DMPP.

REFERENCES

Hypoxia and the Pulmonary Circulation

Differences of opinion exist as to whether the pulmonary arterial hypertension induced by hypoxia is dominantly or wholly due to increased resistance in the pulmonary circuit or to augmented output of the right ventricle. It is becoming apparent that differing conclusions are based partly on technical difficulties in determining cardiac output during anoxia by application of the Fick principle (Nahas and Associates, J. Applied Physiol. 6: 567, 1954).

Recent studies on dogs from the Physiopathological Laboratory of Gand seem to support this conclusion. In addition, when experiments on anesthetized dogs breathing room air are prolonged, pulmonary resistance increases progressively.

When these experimental complications were avoided, it was again found that acute hypoxia (breathing 7 to 10 per cent oxygen mixture) generally increases cardiac output but does not augment pulmonary arterial resistance.

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