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The role of acetylcholine in peptone shock

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Thesis

THE ROLE OF ACETYLCHOLINE IN PEPTONE SHOCK

by

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THE ROLE OF ACETYLCHOLINE IN PEPTONE SHOCK

Introduction

It was the purpose of this thesis to determine the relative importance of the role of acetylcholine in the production of the condition known as peptone shock. A series of acute experiments, using dogs as the experimental animals, was performed to compare the efficacy of various methods of treatment in alleviating this reaction.

De Waele (5) in 1907 first noted the marked similarity between the symptoms of peptone and anaphylactic shock. The essential difference between the two is that a solution of peptones or proteoses produces shock when first injected, whereas an initial dose of antigen, followed by a second injection after a suitable period of time has elapsed, is necessary to produce anaphylaxis. In the signs and symptoms produced, the two are quite similar.

In 1910, soon after the discovery of histamine, Dale and Laidlaw (3) noted the close similarity between anaphylactic and peptone shock and the results produced by an injection of histamine. They postulated that histamine is the active substance producing the signs of anaphylaxis. This theory was not substantiated until 1932, when Dragstedt (6) found, in blood from the inferior vena cava and lymph from the thoracic duct, a substance, appearing during the early stages of anaphylactic shock in dogs, which behaved chemically and physiologically like histamine. It is now generally accepted that histamine plays the major role in both peptone and anaphylactic shock.
With the introduction by Bovet and Staub (2) in 1937 of the first in a series of compounds possessing marked anti-histaminic properties, the importance of histamine in these conditions could be determined. N-Pyridyl-N-benzyl-N', N'-dimethylethlyenediamine hydrochloride, Pyribenzamine, introduced in 1945 by Mayer, Huttrer and Scholz, (15), is a potent antihistaminic drug, and has been widely used in this country. All the drugs which show a marked antagonism to histamine are capable of diminishing the severity of anaphylactic and peptone shock (12).

Several observations have been made which indicate that some other factor(s) may be involved also. In 1936 Went and Lissak (20) observed that the choline content of the isolated sensitized guinea pig heart decreased following the addition of antigen, and that acetylation of the perfusing fluid gave an acetylcholine-like reaction with leech muscle. They also observed that the isolated, sensitized rat heart slows when antigen is added to the perfusion fluid.

Farber and his co-workers (7) found that in 10% of 27 isolated sensitized guinea pig hearts there was an acetylcholine-like substance liberated when antigen was added to perfusion fluid containing physostigmine. Normal hearts did not show such activity.

Wenner and Buhrmester (19) found that the acetylcholine concentration in the blood of rabbits in anaphylactic shock was 1:1,000,000 - 10,000,000, while normal rabbits showed no measurable amounts of acetylcholine activity.

To explain the origin of the increased acetylcholine present in shock, irritation of cholinergic fibres has been postulated (11). But Kuchinski (10) noted that the cholinesterase activity of the serum in cats and dogs
is diminished, both in vitro and in vivo, when peptone is added. In vivo there was a sharp drop in cholinesterase activity on administration of peptone to about 50% of normal, with a gradual rise to 70-75% of normal in one hour. A decrease in cholinesterase activity has also been reported in anaphylactic shock (11).

If acetylcholine plays a role in the production of shock, pretreatment with a substance blocking the effects of acetylcholine should lessen the severity of such reactions.

Auer (1) in 1910 reported that atropine is of some value in anaphylactic shock. Of 25 sensitized guinea pigs treated with atropine, 1-5 mgm. per Kg., before reinjection of the sensitizing serum, 18 or 72% survived, while of 24 similar pigs which did not receive atropine 6 or 25% survived.

Gross (8) has reported that, in dogs, 0.3 Gm./Kg. of Witte's peptone, given intravenously, is the minimal lethal dose. Atropine, 0.2 mgm/Kg., prevented death in all dogs receiving 0.5 Gm. of peptone. Atropine in this dosage did not protect against the lethal effects of 0.6 Gm., however. The number of dogs used was not stated.

On the other hand, in a report by Vallery-Radot, Maurice, and Haltzer, (18) atropine, given in doses of 0.004-0.5 mgm/Kg., did not prevent anaphylactic shock in 7 rabbits. One rabbit receiving 0.5 mgm. and one receiving 1.0 mgm. per Kg. did not show shock. They concluded that atropine did not prevent anaphylactic shock.

Danielopolou (4), however reports that atropine hinders, while eserine aids, the production of anaphylactic shock. Dosages were not given.
The relative importance of acetylcholine in these conditions has not been determined. A series of experiments has been performed in an attempt to compare the beneficial effects of an anti-acetylcholine drug and an anti-histaminic compound in peptone shock.

**Methods**

Mongrel dogs were anesthetized with sodium pentobarbital, given intravenously. The left carotid was exposed, cannulated, and connected with a U-tube mercury manometer modified for use with the electrical recording system described by Maison and Haterius (13). Pulse rates were obtained by palpation. All drugs were administered via the femoral vein, which was exposed for this purpose.

Atropine sulfate, 2.0 mgm./kg. and Pyribenzamine*, 10.0 mgm./Kg., either alone or in combination were used as pre-peptone medication. These were administered intravenously as a 1.0% solution. Ten to 15 minutes were allowed to elapse after the administration of Pyribenzamine before peptone was given. In the dogs receiving atropine, the right vagus was stimulated electrically before and after the injection. In the dosage used, the cardiac vagus was apparently blocked, as the same intensity of stimulus causing a marked fall in blood pressure before atropine had no effect after the drug was given. Five c.c. of a 20% solution (1.0 Gm.) of Witte's peptone intravenously was then injected per kilogram body weight rapidly as possible, using a #22 needle. The time required for the injection was 1.5 to 3 minutes depending upon the total volume used.

*The Pyribenzamine used was supplied by Ciba Pharmaceutical Products, Inc.
Results

Mortality: Three controls, receiving only peptone, died within 5 to 11 minutes after the beginning of the injection. Three vagotomized dogs died also, one 5 minutes, one 20 minutes, and one 90 minutes after peptone. Of three dogs pretreated with atropine, 3 with Pyribenzamine, and 5 with both, none died.

Pulse rates: During the peptone injection, the heart rate in all but one of the animals slowed 4 to 52 beats per minute from the initial rate. Within 2-5 minutes after the injection, the rate increased, in all but 2, to a level greater than that prior to peptone. This is probably indicative of a sympathetic discharge due to the hypotension present. Table 1 is a tabulation of the changes in pulse rates occurring during and after peptone.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Expt. No.</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine- SO₄, 2.0 mgm./Kg.</td>
<td>1</td>
<td>204</td>
<td>180</td>
<td>208</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>188</td>
<td>168</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>188</td>
<td>176</td>
<td>176</td>
</tr>
<tr>
<td>Pyribenzamine, 10 mgm./Kg.</td>
<td>56</td>
<td>164</td>
<td>160</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>168</td>
<td>140</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>130</td>
<td>144</td>
<td>140</td>
</tr>
<tr>
<td>Pyribenzamine, 10 mgm./Kg. and Atropine- SO₄, 2.0 mgm./Kg.</td>
<td>4</td>
<td>160</td>
<td>144</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>190</td>
<td>140</td>
<td>208</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>172</td>
<td>120</td>
<td>184</td>
</tr>
<tr>
<td></td>
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<td>160</td>
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<td>188</td>
</tr>
<tr>
<td></td>
<td>62</td>
<td>184</td>
<td>152</td>
<td>192</td>
</tr>
</tbody>
</table>

Table 1

Pulse rates (A) before, (B) during, and (C) 2-5 minutes after peptone.

Blood pressure: All the dogs, regardless of the type of pretreatment, showed an initial precipitous fall in blood pressure to about 30 millimeters of mercury, beginning within 15 to 30 seconds after commencing the peptone
injection. An example of this fall is seen in Fig. 1. Continuation of the injection evoked a further fall of only 5 to 10 mm. in the treated animals, while the controls showed a progressive decline to zero. In most of the surviving animals the blood pressure began to rise within 4 to 10 minutes, reached a peak in 9 to 16 minutes, declined a few millimeters and then began a secondary more gradual rise within 20 to 30 minutes, reaching a plateau in about one hour. (All time intervals given refer to the time elapsed from the beginning of the peptone injection.) Three animals did not show the initial peak, but exhibited a slow continuous rise until the final plateau. Otherwise they were similar to the remaining animals. A typical example of the blood pressure changes for each of the three methods of treatment is given in Fig. 2.

Since Pyribenzamine potentiates the response to epinephrine (16), the blood pressure at 25 minutes post-peptone, at which time the pressure was at a plateau or just beginning its secondary rise, was used as an indication of the degree of protection afforded by the drugs, as this excluded the effects of the initial sympathetic discharge. Twenty-five minutes was arbitrarily chosen, as with further recovery, the differences between the methods used became less marked. In Table II the blood pressure changes are given.

None of the three methods of treatment prevented the initial fall in blood pressure. Both the height of the initial peak and the pressure at 25 minutes were greater in those treated with Pyribenzamine alone than in those receiving atropine alone. In the dosages used atropine protects dogs from death due to peptone; Pyribenzamine afforded better protection than atropine.
A combination of the two afforded no better protection than Pyribenzamine alone.

The effect upon the blood pressure of injection of peptone, 1.0 Gm/Kg. I.V. Injection begun at A, completed at B. Time interval = 6 seconds. Pressure at A = 140; pressure at B = 28.

Table II. Tabulation of blood pressures (in mm. Hg.) (A) before peptone; (b) lowest pressure reached when peptone given; (C) pressure at crest of initial rise; (D) pressure at 25 minutes after peptone.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Expt. No.</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
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<tr>
<td>Atropine, S04,</td>
<td>1</td>
<td>138</td>
<td>30</td>
<td>60</td>
<td>45</td>
</tr>
<tr>
<td>2.0 mgm./Kg.</td>
<td>52</td>
<td>170</td>
<td>28</td>
<td>58</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>134</td>
<td>18</td>
<td></td>
<td>44</td>
</tr>
<tr>
<td>Pyribenzamine,</td>
<td>56</td>
<td>126</td>
<td>52</td>
<td>122</td>
<td>112</td>
</tr>
<tr>
<td>10 mgm./Kg.</td>
<td>61</td>
<td>142</td>
<td>20</td>
<td>106</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>128</td>
<td>26</td>
<td>56</td>
<td>104</td>
</tr>
<tr>
<td>Pyribenzamine,</td>
<td>4</td>
<td>80</td>
<td>28</td>
<td>112</td>
<td>106</td>
</tr>
<tr>
<td>10 mgm./Kg.</td>
<td>5</td>
<td>68</td>
<td>10</td>
<td>52</td>
<td>44</td>
</tr>
<tr>
<td>and</td>
<td>57</td>
<td>144</td>
<td>24</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Atropine-S04,</td>
<td>59</td>
<td>136</td>
<td>22</td>
<td>74</td>
<td>70</td>
</tr>
<tr>
<td>2.0 mgm./Kg.</td>
<td>62</td>
<td>136</td>
<td>24</td>
<td>74</td>
<td>80</td>
</tr>
</tbody>
</table>

* Showed no initial peak.
Fig. 2. Typical blood pressure changes occurring after administration of peptone 1.0 Gm. per Kg. body weight. Pretreatment: #52 - Atropine sulphate 2.0 mgm/Kg; #61 - Pyribenzamine 10.0 mgm./Kg.; #62 - Pyribenzamine 10.0 mgm/Kg. and atropine sulfate 2.0 mgm/Kg.

Discussion

The failure to prevent the initial fall in blood pressure is consistent with the results of Yonkman, Hays, and Renninck (21) in anaphylactic shock. They, however, reported that the heart is apparently not slowed during the hypotensive phase in anaphylaxis after pretreatment with Pyribenzamine, but this does not agree with our results in peptone shock.

One difficulty to be overcome before accepting the hypothesis that acetylcholine plays a role in the production of peptone shock is that, as
yet, no drug is available which exhibits antagonism to acetylcholine without possessing some antihistaminic action. Similarly all antihistaminic drugs show a certain degree of anti-acetylcholine activity. Various degrees of specificity are possible however. Thus Pyribenzamine is 250 times as potent as atropine against histamine as tested by the aerosol method of producing histamine shock in guinea pigs (9) (14). Atropine is much more effective as an anticholinergic drug than is Pyribenzamine. In our experience 10.0 mgm./Kg. of Pyribenzamine exerted only moderate vagal blockage, as compared with 0.05 mgm./Kg. of atropine required for vagal blocking in the dog (17).

Fig. 2. Effect of vagal stimulation on blood pressure before A and 10 minutes after B pyribenzamine, 10 mgm./Kg. given I.V. The right vagus was stimulated electrically at S. Time intervals = 10 seconds.

To protect 100% of sensitized guinea pigs from lethal anaphylaxis, 0.3 mgm./Kg. of Pyribenzamine must be given (14). The exact dosage required to protect dogs has not been determined, but it seems to be somewhat greater. Thus the protective action of 2.0 mgm./Kg. of atropine in peptone shock can
hardly be attributed to its antihistaminic properties. It seems much more likely that the benefit derived is a result of its anticholinergic activity. No explanation is offered for the observation that a combination of Pyribenzamine and atropine seems to less effective than Pyribenzamine alone.

The participation of acetylcholine in peptone or anaphylactic shock is not accepted generally. Loew (12) states that no conclusive evidence has been offered concerning its role in anaphylaxis. Danielopolou (4), on the other hand, believes that acetylcholine is the most important factor, and assigns a secondary role to histamine. More quantitative comparisons between the antihistaminics and anticholinergic drugs are necessary before the question can be resolved, but the greater degree of protection afforded by Pyribenzamine than by atropine, as evidenced in these experiments by the blood pressure changes, is evidence that while histamine plays the major role in the production of peptone shock, acetylcholine is also concerned.

Conclusions

1) One Gm./Kg. body weight of Witte's peptone administered intravenously in a 20% solution, is fatal in dogs anesthetized with sodium pentobarbital.

2) Atropine sulfate, 2.0 mg./Kg., prevents death from peptone in this dosage.

3) Pyribenzamine, 10.0 mgm./Kg., affords better protection than atropine as determined by rapidity of recovery of blood pressure.

4) A combination of the two drugs affords no better protection than Pyribenzamine alone.

5) These findings support the theory that acetylcholine is concerned in the production of peptone shock, although histamine plays a more important role.
THE ROLE OF ACETYLCHOLINE IN PEPTONE SHOCK
(Abstract)

Anaphylactic shock and the phenomenon produced by the injection of a solution of peptones or proteoses exhibit quite similar signs and symptoms. Both conditions are characterized by a release of histamine, which is accepted to be the major factor in the production of these syndromes. Various drugs have been introduced in the last ten years which possess marked antihistamine activity. All such drugs are capable of diminishing the severity of peptone and anaphylactic shock.

Other factors may also be involved, however. Went and Lissak have reported that the choline content of the isolated, sensitized guinea pig heart decreases following the addition of antigen to the perfusion fluid; Farber et al found that 10% of such hearts liberated an acetylcholine-like substance when antigen was added, while Wenner and Bunmeester report that the acetylcholine concentration of the serum of rabbits in anaphylactic shock is higher than that of normals.

These observations may be explained by the observation by Kucinski that the cholinesterase activity of the serum of animals is decreased to about 50% of normal when peptone is given. The serum cholinesterase is also diminished in anaphylactic shock.

If the release of acetylcholine is concerned in the production of the state of shock, atropine should alleviate the condition. Auer has reported that such is the case in anaphylaxis in guinea pigs, while Gross has reported that atropine is of value in peptone shock. Others have failed to confirm these findings, however.
To compare the efficacy of atropine and Pyribenzamine (N'-pyridyl-N'-benzyl-N-dimethylenediamine·HCl), one of the more potent antihistaminic compounds, and thus compare the relative importance of acetylcholine and histamine in peptone shock, a series of experiments was performed, using dogs anesthetized with sodium pentobarbital. Carotid blood pressure was recorded, and pulse rates were followed. Atropine sulfate, 2.0 mgm./Kg., and Pyribenzamine, 10.0 mgm./Kg., were given as pre-peptone treatment, either alone or in combination. A 20% solution of Witte's peptone, 5.0 c.c./Kg., was then injected via the femoral vein.

Three controls, receiving only peptone, and three vagotomized dogs died. Of three dogs treated with atropine, three with Pyribenzamine, and five receiving both, none died.

During the peptone injection the heart rate slowed in all dogs except one. In 2 to 5 minutes after the injection most of the surviving animals showed an increase in heart rate to a level greater than that prior to the peptone injection.

With 30 seconds after the beginning of the injection, all the dogs showed a precipitous fall in blood pressure to about 30 millimeters of mercury. In those animals which survived, the pressure began to rise in 4 to 10 minutes after the injection, reached a peak in 9 to 16 minutes, declined slightly and then began a slower secondary rise reaching a plateau in about one hour. Those receiving Pyribenzamine showed a greater initial rise and a higher pressure at 25 minutes after peptone than those receiving atropine.

The dogs receiving both drugs varied considerably in their response, but none showed a greater degree of recovery than those receiving Pyribenzamine alone.
...
Our failure to prevent the initial fall in blood pressure is consistent with the reports of others.

The antihistaminic potency of atropine is $1/250$ that of Pyribenzamine. More than 0.3 mgm./Kg. of Pyribenzamine is necessary to protect dogs from death in anaphylaxis. Thus the dosage of atropine used, 2.0 mgm./Kg., can hardly have exerted its beneficial effect in peptone shock through its antihistaminic effects, but did so probably via its anti-acetylcholine activity.

Conclusions

1. Witte's peptone, 1.0 Gm./Kg. body weight, given intravenously as a 20% solution, is fatal in dogs anesthetized with sodium pentobarbital.

2. Atropine sulfate, 2.0 mgm./Kg., prevents death from this dosage of peptone.

3. Pyribenzamine, 10.0 mgm./Kg., affords better protection than atropine, as determined by rapidity of recovery of blood pressure.

4. A combination of the two drugs affords no better protection than Pyribenzamine alone.

5. These findings support the theory that acetylcholine is concerned in the production of peptone shock, although histamine plays a more important role.
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