Effect of Hexamethonium on the Adrenaline Secretion in Response to Acetylcholine of the Denervated Adrenal Gland

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It is now fairly generally accepted that hexamethonium has the blocking effect on the sympathetic ganglion.

As regards its effect on the adrenal medulla, Schachter1) reported that in dogs the hypoglycemic effect of insulin was intensified by the application of hexamethonium. According to him it is probably due to the blocking effect of the latter on the adrenal medulla. In estimating directly the adrenaline secretion rate of the adrenal gland, Yamashita found that in the dog the augmentation in the adrenaline secretion causable by acetylcholine2) and by insulin hypoglycemia3) was greatly diminished by hexamethonium. He concluded that hexamethonium paralyzed the adrenal medullary cells. The above investigations aimed to elucidate the effect of hexamethonium on the innervated adrenal medulla. Little is known, however, of its effect on the denervated gland.

Concerning the effect of hexamethonium on the denervated sympathetic ganglion, Perry and Reinert4) demonstrated in cats that the blocking effect of hexamethonium on the ganglionic response to acetylcholine was abolished by the degenerative section of preganglionic nerve fibers. They used the retraction of the nictitating membrane as the indicator of ganglionic response.

The present study was undertaken to know whether the adrenaline-secretory effect of acetylcholine on the denervated adrenal medulla is suppressed or not by hexamethonium.

Experimental

Methods

Nine dogs, weighing from 6.6 to 13.5 kg., were used. The denervation of the adrenal gland was done by cutting the ipsilateral splanchnic nerves through the lumbar route. All the experiments were performed
under Evipan-sodium anesthesia. The adrenal venous blood was collected through a small cannula inserted into the adrenal vein just lateral to the gland, according to the method of Satake et al. The adrenaline content of the adrenal venous blood specimens was estimated by the Bloor and Bullen's arseno-molybdic acid method, as the reference standard. In most experiments the blood pressure was recorded by the mercury manometer connected with femoral artery. For the intravenous injection of chemicals the saphenous vein was prepared and a small cannula was inserted into the vein.

After atropinizing the animal, acetylcholine chloride (Hoffmann La Roche) was injected intravenously in a dose of 2 mg. per kg. of body weight in 15 seconds. After a while hexamethonium (Bistrium bromide, Squibb) was injected intravenously in a dose of 0.75 mg. per kg. Then, acetylcholine in the same dose as before was injected again. The adrenal venous blood was collected before injections of acetylcholine, as well as during the first and the second 60-second periods after the start of acetylcholine injections.

Results

(A) Experiments in acutely splanchnicotomized dogs.

Experiments were carried out in 3 dogs, whose splanchnic nerves were cut about 2 hours before the onset of experiments. The results are presented in Table I.

In Exp. 1, the adrenaline secretion rate before the first acetylcholine injection was 0.02 μg. per kg. of body weight per minute. On receiving acetylcholine it was accelerated and reached 0.30 μg. in the first 60-second

| Table I |

<table>
<thead>
<tr>
<th>No. of experiment</th>
<th>Body weight and sex</th>
<th>Adrenaline secretion rate (μg.) per kg. per minute</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before hexamethonium</td>
<td>After acetylcholine (sec.)</td>
</tr>
<tr>
<td></td>
<td>Before acetylcholine</td>
<td>0-60</td>
</tr>
<tr>
<td>1</td>
<td>13.5 kg. ♀</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>9.0 kg. ♂</td>
<td>*</td>
</tr>
<tr>
<td>3</td>
<td>6.6 kg. ♂</td>
<td>0.02</td>
</tr>
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</table>

* Immeasurably small amount of adrenaline in adrenal venous blood.
period after the start of injection. In the second 60-second period it returned to 0.03 µg. After injection of hexamethonium the secretion rate was immeasurably small. By the second acetylcholine injection the adrenaline secretion rate was not increased, 0.01 µg. being estimated in the first 60-second period. And in the second 60-second period it was too small to be estimated.

In Exp. 2, the rate of adrenaline secretion before the first acetylcholine injection was immeasurably small. After the injection it increased and reached 0.34 µg. per kg. per minute in the first 60-second period. After hexamethonium the adrenaline secretion rate was 0.03 µg. By the second acetylcholine injection no appreciable increase in the secretion rate was induced, 0.03 µg. and 0.01 µg. being estimated in the first and the second 60-second period, respectively.

In Exp. 3, the adrenaline secretion rate before the first acetylcholine injection was 0.02 µg. per kg. per minute. After the injection it increased to 0.34 µg. in the first 60-second period. It was immeasurably small in the second 60-second period. After hexamethonium the adrenaline secretion rate was too small to be estimated. After the second acetylcholine injection the secretion rate was estimated as 0.04 µg. and 0.03 µg. in the first and the second 60-second period, respectively.

(B) Experiments in chronically splanchnicotomized dogs

Six dogs were used. In 3 dogs (Exps. 4–6) the splanchnic nerves were cut 2 weeks and in 3 others (Exps. 7–9) 3 weeks prior to experiments. The results are shown in Table II.

In Exp. 4, the pre-injection control secretion rate of adrenaline was 0.01 µg. per kg. per minute. On receiving acetylcholine, the adrenaline secretion rate increased to 0.28 µg. in the first 60-second period after the start of injection. And in the second 60-second period it decreased to 0.02 µg. After hexamethonium injection, the adrenaline secretion rate before the second acetylcholine injection was immeasurably small. After the acetylcholine injection the rate of adrenaline secretion increased and reached 0.19 µg. in the first 60-second period. Then it decreased to 0.03 µg.

Almost the same results were obtained in Exp. 5. The rate of adrenaline secretion before the first acetylcholine injection was 0.03 µg. per kg. per minute. After the injection of acetylcholine it increased and reached 0.38 µg. in the first 60-second period. In the next 60-second period it was measured as 0.10 µg. After application of hexamethonium the adrenaline secretion rate was too small to be estimated. By the second acetylcholine injection the secretion rate was increased to 0.14 µg. in the first 60-second period. It was 0.05 µg. in the second 60-second period.

In Exp. 6, the adrenaline secretion rate before the first acetylcholine
TABLE II

Effect of Hexamethonium on the Augmented Adrenaline Secretion of the Adrenal Gland Causable by Acetylcholine in Chronically Splanchnicotomized Dogs

<table>
<thead>
<tr>
<th>No. of experiment</th>
<th>Body weight and sex</th>
<th>Weeks after splanchnicotomy</th>
<th>Adrenaline secretion rate (µg.) per kg. per min.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before hexamethonium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before acetylcholine (sec.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0-60</td>
</tr>
<tr>
<td>4</td>
<td>8.9 kg. ♀</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>11.0 kg. ♂</td>
<td>2</td>
<td>0.03</td>
</tr>
<tr>
<td>6</td>
<td>9.9 kg. ♂</td>
<td>2</td>
<td>0.03-0.04</td>
</tr>
<tr>
<td>7</td>
<td>9.4 kg. ♀</td>
<td>3</td>
<td>0.03-0.04</td>
</tr>
<tr>
<td>8</td>
<td>7.5 kg. ♀</td>
<td>3</td>
<td>*</td>
</tr>
<tr>
<td>9</td>
<td>8.2 kg. ♀</td>
<td>3</td>
<td>0.02-0.03</td>
</tr>
</tbody>
</table>

* Immeasurably small amount of adrenaline in adrenal venous blood.

Injection was 0.03–0.04 µg. per kg. per minute. By the first acetylcholine injection it was increased to 0.99 µg. in the first 60-second period. In the second 60-second period it returned to the pre-injection secretion rate. Hexamethonium was then injected. The adrenaline secretion rate before the second acetylcholine injection was 0.02 µg. After injection of acetylcholine it increased to 0.14 µg. in the first 60-second period. In the next 60-second period it decreased to 0.03 µg.

In Exp. 7, the rate of adrenaline secretion before the first injection of acetylcholine was 0.03–0.04 µg. per kg. per minute. After the injection it was accelerated and estimated as 0.44 µg. and 0.11 µg. in the first and the second 60-second period, respectively. After administration of hexamethonium and before the second acetylcholine injection, the rate of adrenaline secretion was 0.01 µg. After the second acetylcholine injection it increased, 0.26 µg. and 0.06 µg. being estimated in the first and the second 60-second period, respectively.

Similar results were obtained in Exp. 8. The adrenaline secretion rate before the first acetylcholine injection was immeasurably small. After injection of acetylcholine it increased to 0.42 µg. per kg. per minute in the first 60-second period. In the second 60-second period it was estimated at 0.03 µg. After administration of hexamethonium, acetylcholine injection was made again. Before the second acetylcholine injection the adrenaline secretion rate was 0.02 µg. After the injection of acetylcholine it increased and reached 0.22 µg. in the first 60-second period. In the next...
60-second period it was estimated at 0.06 μg.

In Exp. 9, the adrenaline secretion rate before the first acetylcholine injection was 0.02-0.03 μg. per kg. per minute. By the injection of acetylcholine it was increased and reached 0.82 μg. in the first 60-second period after the start of injection. In the next 60-second period it was 0.08 μg. After hexamethonium and before the second injection of acetylcholine the adrenaline secretion rate was 0.02 μg. On injecting acetylcholine it increased and was estimated as 0.20 μg. and 0.09 μg. in the first and the second 60-second period, respectively.

**DISCUSSION**

In Exps. 1–3, in which the section of the splanchnic nerves was made about 2 hours before the onset of experiments, the secretion rates of the first 60-second periods after the first and the second acetylcholine injection were 0.30–0.34 μg. and 0.01–0.04 μg. per kg. per minute, respectively. The ratio of the latter to the former was on the average about 8:100. In experiments of Yamashita2) with the innervated adrenal gland, the average ratio was approximately 5:100. Thus it is evident that the effect of hexamethonium on the acutely denervated adrenal gland is almost the same as that on the innervated gland.

On the other hand, in Exps. 4–6, in which the splanchnic nerves were cut 2 weeks before experiments, the adrenaline secretion rates after the first and the second acetylcholine injection were 0.28–0.99 μg. and 0.14–0.19 μg., respectively. The mean ratio of the latter to the former was about 40:100. In Exps. 7–9, in which the splanchnic nerves were cut 3 weeks before experiments, the secretion rates after the first and the second acetylcholine injection were 0.42–0.82 μg. and 0.20–0.26 μg., respectively. The ratio of the latter to the former was on the average 45:100. Thus it is deductible that in the chronically denervated adrenal gland a considerable increase in the adrenaline secretion is causable by acetylcholine even after administration of hexamethonium.

It is concluded that the paralyzing effect of hexamethonium on the adrenal medullary response to acetylcholine which is not affected by the acute denervation of the adrenal gland, is reduced definitely, if not completely, by the chronical denervation.

**SUMMARY**

Nine dogs, anesthetized with Evipan-sodium, were used in the experiments. The adrenal venous blood was collected by the lumbar route method of Satake et al. and its adrenaline content was estimated chemically by the method of Bloor and Bullen.
In dogs, whose splanchnic nerves were cut about 2 hours before the onset of the experiments, the adrenaline secretion rate was increased markedly by the intravenous injection of acetylcholine before administering hexamethonium. After hexamethonium, however, no definite increase in the secretion rate was elicited by acetylcholine.

In dogs, whose splanchnic nerves were cut 2 or 3 weeks before experiments, a considerable increase in the secretion rate was induced by injection of acetylcholine even after administering hexamethonium, although it was smaller than that before hexamethonium.

Thus it may be reasonable to conclude that the suppressive effect of hexamethonium on the adrenal medullary response to acetylcholine is definitely reduced by the chronic denervation of the adrenal gland, but not by the acute denervation.

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References