Potentiation of Smooth Muscle Contraction by Adrenal Steroids

By Noble O. Fowler, M.D., and Nora H. F. Chou, M.S.

Furchgott, Bhadrakom, and Helmer have employed spirally cut strips of rabbit aorta in a bioassay method for estimation of urinary levels of catechol amines. In attempting to use this method for determination of adrenal venous plasma levels of epinephrine and norepinephrine, the possibility arose that adrenal steroids might alter the contraction of rabbit aortic smooth muscle produced by these catechol amines. This paper describes the effect of five adrenal steroids—hydrocortisone, corticosterone, aldosterone, androsterone, and dehydroisoandrosterone—upon the contraction of the rabbit aortic strip produced by levarterenol (1-noradrenaline).

Although none of the steroids tested produced contraction of the rabbit aortic strip alone, each steroid produced significant potentiation of the contraction of the rabbit aortic strip resulting from levarterenol.

Methods

The experimental preparations were essentially the same as described by Helmer. The spirally cut rabbit aortic strip was mounted in a 20-ml-capacity muscle chamber containing Krebs’ bicarbonate solution of the following composition: In 1 L. of double distilled water were dissolved 6.92 Gm. NaCl; 0.708 Gm. KCl; 0.282 Gm. CaCl₂; 0.162 Gm. KH₂PO₄; 0.144 Gm. MgSO₄·7H₂O; 2.21 Gm. NaHCO₃; and 2 Gm. dextrose. Ninety-five per cent O₂ and 5 per cent CO₂ were bubbled through the solution to maintain a pH of 7.4 ± 0.05. To each 20-ml. muscle bath was added 0.5 mg. disodium versenate, as suggested by Helmer. The muscle chamber was immersed in a constant temperature water bath maintained at 37.5 ± 0.1 C.

Results

The solution to be tested was added to the chamber and the contraction of the strip was magnified 10 times and recorded by a heated stylus upon heat-sensitive paper on a kymograph. The kymograph speed was usually 72 mm./hr. but was occasionally 144 mm./hr. Levarterenol bitartrate dissolved in 0.01 N HCl was added to the bath in amounts of 0.1 to 0.2 μg. and the contraction recorded. After contraction was maximal, the muscle chamber was irrigated with fresh Krebs’ bicarbonate solution, causing a return of the stylus to the baseline.

After three or more consistent contractions were recorded in response to a constant amount of levarterenol, the adrenal steroid to be tested was dissolved in 0.1 to 5 ml. of Krebs’ solution, preheated to 37.5 C. and added to the bath. The previous amount of levarterenol was then added and the amplitude of contraction recorded. The muscle chamber was then irrigated, and the response to the previous amount of levarterenol was recorded until three or more contractions were of an amplitude similar to the control contractions. It was essential that the steroid to be tested be dissolved in Krebs’ bicarbonate solution rather than distilled water, since distilled water alone was found to be capable of producing contraction of the rabbit aortic strip when added to the bath.

The results are summarized in the table. Corticosterone was capable of producing potentiation in the smallest amounts of any of the five steroids tested. Potentiation was observed in amounts as small as 1 μg., and consistently with amounts of 5 μg. The maximum potentiation observed was 36.1 per cent and was seen with 25 μg. Amounts up to 100 μg. produced no further potentiation. Figure 1 shows the potentiation of smooth muscle contraction produced by 25 μg. of corticosterone. Levarterenol in amounts of 0.1 μg. produced a 35-mm. contraction. After addition of corticosterone, the contraction increased to 48 mm. As was often seen, the first few contractions after corticosterone were somewhat higher than the control, but the fourth contraction after corticosterone showed a return.
Table 1
Potentiation of Rabbit Aortic Smooth Muscle Contraction by Adrenal Steroids

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Amounts tested µg.</th>
<th>Minimum producing potentiation</th>
<th>Minimum producing consistent potentiation</th>
<th>Maximum potentiation per cent</th>
<th>Amount producing maximum effect</th>
<th>Number of strips tested</th>
<th>Number of tests performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosterone</td>
<td>1—100</td>
<td>1</td>
<td>5</td>
<td>36.1</td>
<td>25</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>2—100</td>
<td>2</td>
<td>20</td>
<td>74.2</td>
<td>100</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Aldosterone acetate</td>
<td>2—300</td>
<td>200</td>
<td>200</td>
<td>50</td>
<td>300</td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>Androsterone</td>
<td>20—300</td>
<td>50</td>
<td>50</td>
<td>45.8</td>
<td>300</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Dehydroisoandrosterone</td>
<td>20—100</td>
<td>20</td>
<td>20</td>
<td>42.8</td>
<td>50</td>
<td>5</td>
<td>13</td>
</tr>
</tbody>
</table>

to control amplitude. Twenty µg. of corticosterone alone produced no contraction of the muscle strip.

Hydrocortisone was tested in amounts of 2 to 100 µg. As shown in the table, 2 µg. occasionally produced potentiation, but 20 µg. was the smallest amount producing consistent potentiation. The maximum potentiation observed was 74.2 per cent when 100 µg. was employed. Figure 2 illustrates potentiation of smooth muscle contraction by hydrocortisone. Levarterenol in amounts of 0.2 µg. produced a contraction of 33 mm. When 100 µg. of hydrocortisone was added to the bath, the amplitude of contraction produced by the same amount of levarterenol was 52 mm. The next contraction produced by levarterenol was still somewhat higher than control, but the following contraction had returned to control amplitude. Hydrocortisone alone in amounts of 100 µg. produced no contraction of the smooth muscle strip.

As shown in the table, aldosterone acetate was the weakest potentiator tested. Aldosterone acetate was tested in amounts of 2 to 300 µg. No potentiation was observed until 200 µg. was used. Maximum potentiation was 50 per cent when 300 µg. was employed. In smaller amounts, some depression of contraction was often observed with aldosterone acetate. Five hundred µg. of aldosterone acetate alone produced no contraction of the muscle strip.

Androsterone was tested in amounts from 20 to 300 µg., as shown in the table. Potentiation was observed only in amounts from 50 µg. upward. Maximum potentiation was 45.8 per cent when 300 µg. was used. Five hundred µg. of androsterone alone produced no contraction of the smooth muscle strip.

As shown in the table, dehydroisoandrosterone was tested in amounts from 20 to 100 µg. Maximum potentiation was 42.8 per cent and was produced by the addition of 50 µg. to the bath. Consistent potentiation, however, was observed with 20 µg. Five hundred µg. of dehydroisoandrosterone alone produced no contraction of the muscle strip.

**Discussion**

The importance of the adrenal cortex in control of vasomotion was shown by Fritz and Levine. These authors studied the blood vessels in the meso-appendix of adrenalectomized rats and found an impairment of constriction in response to levarterenol. Topical application of aqueous adrenal cortical extract restored the sensitivity of these vessels to levarterenol.

Bohr, Brodie, and Chou showed that the addition of desoxy corticosterone to a bath containing a rabbit aortic strip consistently potentiated the contractile response of the smooth muscle when epinephrine was used as the agent to produce contraction. They also showed that increasing the potassium concentration or lowering the sodium concentration of the bath consistently increased the amplitude of the muscle contraction produced by...
Potentiation of rabbit aortic strip contraction by corticosterone. Amplitude of contraction produced by 0.1 μg levaterenol was 35 mm. After addition of 25 μg corticosterone to the bath, the amplitude of contraction produced by 0.1 μg levaterenol increased to 48 mm.

Figure 1

Potentiation of rabbit aortic strip contraction by hydrocortisone. Amplitude of contraction produced by 0.2 μg levaterenol was 33 mm. After addition of 100 μg hydrocortisone to the bath, the amplitude of contraction produced by 0.2 μg levaterenol increased to 52 mm.

Figure 2

Potentiation of rabbit aortic strip contraction by androsterone. Amplitude of contraction produced by 0.15 μg levaterenol was 40 mm. After addition of 50 μg androsterone to the bath, the amplitude of contraction produced by 0.15 μg levaterenol increased to 54 mm. Note the persistence of potentiation for the next three contractions.

Figure 3

adrenaline. These authors stated that desoxycorticosterone is known to interfere with potassium transport into the cell; thus, its mechanism of potentiation may be due to an effect upon the transmembrane potassium gradient. Emele and Bonycastle demonstrated an inotropic effect of corticosterone upon the electrically driven cat papillary muscle. Conway and Hingerty showed that desoxycorticosterone inhibited sodium excretion and potassium uptake of sodium yeast cells. Stolkowski and Reinberg showed that desoxycorticosterone decreased both uptake and exit of K⁺ in the isolated frog gastrocnemius. The effect upon uptake was more pronounced, leading to a decrease in intracellular potassium. Friedman, Jamieson, and Friedman showed that lowering the sodium concentration of Krebs-Henseleit solution containing the rat colon increased its contractile response to carbachol. These authors believed that the transmembrane gradient of sodium was an important factor in determining smooth muscle contractility. In a recent study of human bulbar conjunctival vessels, Reis demonstrated potentiation of levaterenol-induced vasoconstriction by cortisone, hydrocortisone, Δ¹ cortisone, and desoxycorticosterone acetate.

Our results differ from those of Heineman and Danowski who reported potentiation of rabbit aortic smooth muscle contraction with hydrocortisone only when plasma was first added to the bath. These authors used epinephrine rather than norepinephrine to produce muscle contraction. The bath used by these workers contained only one-half the potassium concentration used in our experiments. These workers employed only 0.01 to 0.04 μg of epinephrine in their studies, where we used 0.1 to 0.2 μg of levaterenol. Except for these differences, no reason for the discrepancies in our results can be postulated.

The present studies demonstrate a consistent potentiating effect of the five adrenal steroids tested upon levaterenol-produced aortic smooth muscle contraction. The weaker potentiating effect of aldosterone may have been due to the fact that it alone was tested as the acetate; we have been unable to obtain free aldosterone to confirm or negate this possibility. Since no studies were made of sodium or potassium flux across the cell membranes, it is impossible to state whether an alteration in transmembrane gradients of
these two cations was responsible for observed effects. The works cited above indicate that this is a distinct possibility. However, Conway and Hingerty \cite{11} found no difference in the exit rates of \( \text{Na}^{2+} \) and \( \text{K}^{+} \) from the sartorius muscles of normal and cortisol-injected frogs. Other possible mechanisms of these effects, such as an effect on carbohydrate metabolism or on high energy phosphate bonds, must remain purely speculative.

Walker and associates \cite{12} found the concentration of 17-hydroxycorticosteroids in adrenal venous blood of dogs to be from 1.2 to 4.6 \( \mu \text{g}/\text{ml.} \), with concentration as high as 9.6 \( \mu \text{g}/\text{ml.} \) after bleeding. Plasma concentrations should be approximately twice as great. We found consistent potentiation of smooth muscle contraction by hydrocortisone in a concentration of one \( \mu \text{g.} \) per ml. of muscle bath. Since the output of other adrenal steroids may be also increased by bleeding, it seems likely that the potentiating effect of these steroids on smooth muscle contraction could, under these circumstances, interfere with adrenal venous blood catechol amine determinations by this bioassay method. The likelihood of such interference would be reduced if plasma catechol amines were at a high level, so that 0.1 ml of plasma could be used in the bioassay.

**Summary**

The rabbit aortic strip preparation of Furchgott was used to test the effect of adrenal steroids upon the muscle contraction produced by 0.1 to 0.2 \( \mu \text{g.} \) of levarterenol. The steroids tested were corticosterone, hydrocortisone, aldosterone acetate, androsterone, and dehydroisoandrosterone. Amounts of 1 to 300 \( \mu \text{g.} \) were added to the 20-ml muscle bath. Potentiation was produced by each steroid; the percentage increase of amplitude of contraction was from 36.1 to 74.2 per cent. Corticosterone produced consistent potentiation in amounts as small as 5 \( \mu \text{g.} \); 200 \( \mu \text{g.} \) of aldosterone acetate was required to produce potentiation. Maximum potentiation was produced by hydrocortisone. The observed potentiation may be due to an effect of adrenal steroids upon transcellular sodium and potassium gradients.

**Acknowledgment**

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**References**

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NOBLE O. FOWLER and NORA H.F. CHOU

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