Mechanism of Hydrocortisone Potentiation of Responses to Epinephrine and Norepinephrine in Rabbit Aorta

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ABSTRACT
Hydrocortisone potentiated responses of rabbit aortic strips to catecholamines (epinephrine, norepinephrine, noradrenalin, isoproterenol) but not to amines lacking the catechol nucleus (phenylephrine, synephrine, methoxamine). Contractions in response to epinephrine were increased much more than those to norepinephrine. Neither the presence of cocaine nor pretreatment of the rabbits with reserpine impaired the potentiating action of hydrocortisone. Experiments with the oil immersion technique (to prevent loss of amine by diffusion from the tissue) demonstrated that hydrocortisone reduced the rate at which aortic strips inactivated epinephrine, apparently by inhibiting catechol-O-methyl transferase (COMT). Known inhibitors of COMT (U-0521, tropolone, pyrogallol) potentiated responses of aortic strips to epinephrine much more than to norepinephrine and also enhanced responses to isoproterenol and noradrenalin to the same extent as did hydrocortisone. Known inhibitors of COMT consistently abolished the enhancing effects of hydrocortisone without materially interfering with potentiation produced by cocaine which is mediated through an independent mechanism unrelated to amine inactivation. Hydrocortisone also abolished the enhancing effects of known COMT inhibitors. It is concluded that hydrocortisone enhances the responses of vascular smooth muscle to epinephrine and norepinephrine by inhibiting a major enzymatic pathway for the inactivation of these amines.

ADDITIONAL KEY WORDS

- aortic strips
- catecholamine inactivation
- catechol-O-methyl transferase
- cocaine
- smooth muscle
- reserpine
- oil immersion technique
- adrenal cortical steroids

The adrenal cortical steroids enhance responses of vascular smooth muscle to epinephrine and norepinephrine both in vivo and in vitro. Zweifach et al. (1) found that injection of cortisone, or stimulation of the adrenal cortex by adrenocorticotrophin, increased the sensitivity of the mesenteric vascular bed of the rat to topically applied epinephrine. Similarly, Schayer (2) reported that catecholamines enhanced contractions of smooth muscle of the microcirculation within minutes after systemic injection or local application of a soluble glucocorticoid.

Others have observed that responses of the conjunctival vascular bed of rabbits and man to norepinephrine were potentiated 15 minutes after local application of hydrocortisone or cortisone (3, 4).

Several reports indicate that hydrocortisone potentiates the pressor effects of epinephrine but not that of norepinephrine in the cat, rabbit, and dog (5, 6). In agreement with these findings, Kadowitz and Yard (7) observed that the blood vessels of the denervated and pump-perfused hindquarters of cats show an increased constriction in response to epinephrine but not to norepinephrine after hydrocortisone. However, in-vitro preparations of arterial smooth muscle show increased responsiveness to both epinephrine and norepinephrine within several minutes after exposure to hydrocortisone (6, 8, 9).
Various theories have been proposed to account for the potentiation of responses to sympathomimetic amines by hydrocortisone. Besse and Bass (6) suggested that hydrocortisone modified the interaction of epinephrine and norepinephrine with the alpha receptors of vascular smooth muscle. Schayer (2) proposed that hydrocortisone acted to inhibit an intrinsic dilator substance in smooth muscle of the microcirculation. Potentiation by beta-receptor blockade was ruled out by Kadowitz and Yard (7). The present study is a further investigation of the mechanism by which hydrocortisone enhances the responses of isolated strips of arterial smooth muscle to certain sympathomimetic amines.

Methods

Helically cut strips of rabbit thoracic aorta were prepared according to the method of Furchgott (10). The aqueous medium was Krebs-Henseleit (Krebs) solution containing disodium EDTA (0.01 g/liter). The strips, about 2.0 by 23 mm, were placed under a tension of 2 g and allowed to relax for 90 minutes in Krebs solution before drug testing. Contractions were recorded isotonically with 6.8 X magnification on a slowly moving (usually 1.8 mm/min) kymograph paper. All experiments were performed at 37°C in muscle baths of approximately 15 ml working volume. Concentrations of l-norepinephrine and l-epinephrine bitartrates, l-phenylephrine, nordebrin (Cobefrine), methoxamine and tyramine hydrochlorides and l-phenylephrine were expressed as the base. Cocaine hydrochloride and iproniazid phosphate are expressed as the salts.

Hydrocortisone was used both as the free alcohol (Mann) and the 21-sodium succinate (Solu-Cortef, Upjohn). Throughout this paper hydrocortisone refers to the free alcohol unless it is specifically stated that the 21-sodium succinate derivative was used. In experiments in which aortic strips were treated with hydrocortisone before exposure to an agonist, the muscle chambers were filled by pipette with warmed (37°C) Krebs solution containing the hydrocortisone dissolved to the appropriate concentration. In all other experiments hydrocortisone was first dissolved in a small volume of propylene glycol and added directly to the muscle chambers. The standard volume of propylene glycol added was 0.03 ml; in preliminary experiments it was without effect on the basal tone of aortic strips or on their amplitude of response to a variety of agonists. Hydrocortisone 21-sodium succinate is very soluble in water, and was diluted to the appropriate concentration (calculated as the alcohol) in distilled, demineralized water and usually added to the muscle chambers in a volume of 0.045 ml.

Reserpine powder was dissolved in 10% ascorbic acid. Rabbits were injected intramuscularly with 3 to 5 mg/kg, 18 to 24 hours before death. Fresh stock solutions of all drugs were made every few days and were stored at 8°C. All catecholamines were dissolved in acid saline (0.9% NaCl, 0.01N HCl).

Flasks containing the mineral oil (liquid petrolatum, U.S.P., 40 centistokes) were kept at 37°C in a water bath and constantly bubbled with 95% O2-5% CO2. A flow of the O2-CO2 mixture through the muscle baths was maintained when they were filled with either the Krebs solution or oil. The tissues were immersed in oil after a given response had reached a stable plateau, by draining the aqueous medium from the bath and rapidly refilling it with mineral oil, without an intervening wash of the tissue. This trapped a fixed quantity of drug in the tissue. The rate of relaxation of aortic strips in oil can then be equated with intrinsic inactivation of drug involved in the response (11). The possibility must be considered that compounds with high lipid solubility could escape from the tissue aqueous phase during oil immersion. However, contractions in response to methoxamine (a sympathomimetic which is less polar and more lipid soluble than most smooth muscle stimulants) were sustained for over 1 hour in oil, indicating little loss of drug to the external phase (11). In the experiments reported below, hydrocortisone was used as the highly water-soluble 21-sodium succinate derivative to minimize its loss from the tissue aqueous phase during oil immersion.

Evidence for the adequate oxygenation of the tissue during oil immersion, the lack of accumulation of toxic metabolites, and the absence of any pharmacologic action of the oil itself has been previously presented (11). Contractions in response to tyramine, which are particularly sensitive to O2 deprivation, were usually maintained for over 60 minutes in oil (after inhibition of monoamine oxidase) but were reversibly inhibited by changing the gas mixture from 95% O2-5% CO2 to 95% N2-5% CO2. Exposure of quiescent aortic strips to oil for 60 minutes did not significantly alter basal tone nor the amplitude of response to a standard concentration of norepinephrine administered about 30 seconds after return to Krebs solution. These results indicated that there were no apparent
deleterious effects of oil immersion on any factor affecting contractile performance.

Monoamine oxidase (MAO) in the aortic strip was inhibited by adding iproniazid to the muscle chambers, to give a final concentration of 1 or $2 \times 10^{-4}$ g/ml (12, 13). After a 30-minute exposure to the inhibitor, the strips were washed frequently for an additional 30 minutes before drug testing. Catechol-O-methyl transferase (COMT) was inhibited with pyrogallol ($3 \times 10^{-5}$ g/ml), tropolone (1 or $3 \times 10^{-5}$ g/ml) or U-0521 (3'-4'-dihydroxy-2-methyl propiophenone, Upjohn) ($1 \times 10^{-6}$ g/ml) (14-16). These concentrations produced maximal effects. Higher concentrations occasionally depressed the response to epinephrine. Evidence for the specificity and completeness of the procedures used to inhibit mechanisms of amine inactivation in aortic strips exposed to oil has been previously presented (17, 18) as have other details of the procedures employed (11). Mean values are presented with their standard errors and were compared by Student's t-test. Differences with $P$ values of 0.05 or less were considered significant.

**Results**

**EFFECT OF HYDROCORTISONE ON RESPONSES TO EPINEPHRINE**

To obtain an accurate assessment of the effects of hydrocortisone on responses to epinephrine and other sympathomimetic amines, contractions were produced by a concentration of agonist on the steep portion of the dose-response curve. In this region of the curve small changes in the effective drug concentration produce large changes in the amplitude of response. After a stable plateau was reached, usually by 10 to 20 minutes, hydrocortisone was added to the muscle chamber. A 15-minute exposure of quiescent aortic strips to hydrocortisone ($1 \times 10^{-5}$ g/ml) had no effect on basal tone but clearly increased the amplitude of response to epinephrine (Fig. 1a). Preliminary experiments demonstrated that a concentration of $1 \times 10^{-5}$ g/ml hydrocortisone produced

![FIGURE 1](http://circres.ahajournals.org/)

*Effects of hydrocortisone (HC) on responses of aortic strips contracted by various sympathomimetic amines. (a) Upper trace shows potentiation of response to epinephrine (E); lower trace shows lack of effect of hydrocortisone alone. (b) Lack of potentiation of response to phenylephrine (PE). (c) Potentiation of response to norepinephrine (NE). (d) Lack of potentiation of response to synephrine (SY).*

near maximal to maximal enhancement of responses to epinephrine. The potentiation produced by hydrocortisone was exerted rapidly, and was sustained until washout of the muscle chambers. Strips recontracted by epinephrine or other agonists after washout and recovery of basal tone responded to hydrocortisone with an increase in tension which did not appear to differ from that observed initially. Hydrocortisone 21-sodium succinate gave similar results, but a concentration of $3 \times 10^{-5}$ g/ml was required for comparable potentiation.

Hydrocortisone potentiated the responses of strips contracted by epinephrine ($1 \times 10^{-8}$ g/ml) a mean of 18.7% ± 1.8 (SE) (Table 1). This was equivalent to increasing the bath concentration of epinephrine to $2.5 \times 10^{-8}$ g/ml. Neither the presence of cocaine ($1 \times 10^{-5}$ g/ml), which potentiated responses to epinephrine, nor pretreatment of rabbits with reserpine to deplete endogenous stores of catecholamines, interfered with the potentiating action of hydrocortisone. For example, the responses of 12 reserpine-pretreated strips to epinephrine ($1 \times 10^{-8}$ g/ml) were increased 16.8 ± 1.1% by hydrocortisone, an effect not significantly different from that found in untreated strips. The findings with cocaine and reserpine are in agreement with those of Besse and Bass (6).

**EFFECT OF HYDROCORTISONE ON RESPONSES TO OTHER SYMPATHOMIMETIC AMINES**

Responses to norepinephrine ($1 \times 10^{-8}$ g/ml) were increased much less than those to epinephrine in the same concentration (Table 1). The potentiation produced by hydrocortisone was equivalent to increasing the bath concentration of norepinephrine to $1.3 \times 10^{-8}$ g/ml. Responses of aortic strips to noradrenalin and tyramine were also enhanced by hydrocortisone but those to phenylephrine, synephrine and methoxamine were unchanged. These findings are summarized in Table 1; typical traces obtained with several of the drugs are shown in Figure 1. In 6 aortic strips from animals treated with reserpine, the responses to tyramine ($3 \times 10^{-5}$ g/ml) were depressed by hydrocortisone ($1 \times 10^{-6}$ g/ml) and the 21-sodium succinate derivative ($3 \times 10^{-5}$ g/ml).

**EFFECT OF COMT INHIBITION ON POTENTIATION OF RESPONSES TO EPINEPHRINE BY HYDROCORTISONE**

The potentiation produced by hydrocortisone appeared to be selective for responses to amines containing the catechol nucleus. Since the major mechanism for the inactivation of epinephrine and norepinephrine in aortic strips is O-methylation (18), the pos-

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**TABLE 1**  
**Effect of Hydrocortisone on the Response of Aortic Strips to Sympathomimetic Amines**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Concentration (g/ml)</th>
<th>No. strips</th>
<th>Contraction amplitude (mm)*</th>
<th>Increment after hydrocortisone (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>$3 \times 10^{-8}$</td>
<td>16</td>
<td>22.2 ± 1.3</td>
<td>5.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^{-8}$</td>
<td>25</td>
<td>29.1 ± 2.1</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>$3 \times 10^{-8}$</td>
<td>10</td>
<td>22.9 ± 2.7</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^{-8}$</td>
<td>7</td>
<td>37.9 ± 5.0</td>
<td>2.8 ± 0.6</td>
</tr>
<tr>
<td>Noradrenalin</td>
<td>$3 \times 10^{-8}$</td>
<td>6</td>
<td>11.5 ± 2.1</td>
<td>2.8 ± 0.9</td>
</tr>
<tr>
<td>Tyramine</td>
<td>$3 \times 10^{-6}$</td>
<td>8</td>
<td>15.9 ± 1.8</td>
<td>4.8 ± 0.8</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>$5 \times 10^{-8}$</td>
<td>9</td>
<td>10.2 ± 1.9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^{-7}$</td>
<td>11</td>
<td>17.0 ± 1.7</td>
<td>0</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>$1 \times 10^{-8}$</td>
<td>7</td>
<td>18.1 ± 1.2</td>
<td>0</td>
</tr>
<tr>
<td>Synephrine</td>
<td>$5 \times 10^{-6}$</td>
<td>7</td>
<td>8.9 ± 1.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^{-6}$</td>
<td>13</td>
<td>15.7 ± 1.8</td>
<td>0</td>
</tr>
</tbody>
</table>

Hydrocortisone was added to the muscle chambers after responses to agonists had reached stable plateau values. The values shown for methoxamine include those obtained in reserpine-pretreated strips.

*Values are means ± SE.
sibility that hydrocortisone inhibited COMT was explored with the aid of known enzyme inhibitors. Forty-four strips from 7 aortas pretreated with reserpine were initially tested for their sensitivity to epinephrine. In most of the experiments, strips cut from the same aorta were used for all treatment groups to reduce variability, and the initial mean sensitivities of all groups to epinephrine did not differ significantly. After washout and recovery of basal tone the strips were exposed to the COMT inhibitor U-0521 or to hydrocortisone, both in a concentration of $1 \times 10^{-5}$ g/ml, or to both. Ten minutes later, without washout of the muscle chambers, all strips were contracted by cumulatively increasing concentrations of epinephrine. The log concentration-response curves are shown in Figure 2. The responses to epinephrine were potentiated significantly (about threefold) by both hydrocortisone and U-0521. The combination of both agents produced no greater potentiation than did inhibition of COMT alone.

**FIGURE 2**

Effects of U-0521 and hydrocortisone (HC) on responses of aortic strips to cumulative concentrations of epinephrine. Treatment conditions are described in text. Number of preparations represented by each curve is given in parentheses and standard errors of means are shown by vertical bars wherever they do not interfere with clarity.
In additional experiments hydrocortisone or the 21-sodium succinate derivative and U-0521 were added to the chambers of strips already contracted by epinephrine (3 × 10⁻⁹ or 1 × 10⁻⁸ g/ml). Typical records from one of these experiments are shown in Figure 3a. The addition of U-0521 did not further potentiate the responses of strips contracted by epinephrine and exposed to hydrocortisone 21-sodium succinate (right) and in several strips produced a slight, but transient depression of response amplitude. Similarly hydrocortisone 21-sodium succinate did not clearly increase the amplitude of response of strips contracted by epinephrine and exposed to U-0521 (left). In a few strips, a slight and gradual increase in response amplitude, less than 1 mm, was observed. Similar results were obtained with hydrocortisone (free alcohol).

In other experiments, aortic strips were exposed to a COMT inhibitor for 10 to 20 minutes, followed by epinephrine (3 × 10⁻⁹ or 1 × 10⁻⁸ g/ml) and treatment with hydrocortisone (1 × 10⁻⁵ g/ml) or the 21-sodium succinate derivative (3 × 10⁻⁵ g/ml). The inhibitors tropolone (1 or 3 × 10⁻⁵ g/ml), pyrogallol (3 × 10⁻⁶ g/ml) and U-0521 (1 × 10⁻⁵ g/ml) abolished the enhancing effects of both forms of hydrocortisone in a total of 21 untreated and reserpine-pretreated strips. Similarly, pretreatment of strips with hydrocortisone or the 21-sodium succinate derivative markedly reduced and usually completely abolished the enhancing effect of COMT inhibitors added after responses to epinephrine had reached a plateau. Inhibition of COMT did not materially alter the potentiation produced by cocaine, which involves a mechanism independent of amine inactivation (17-19). Typical traces from several of the above experiments are shown in Figures 3b, 4a and 5.
Effects of COMT inhibition on enhancement of responses to sympathomimetic amines by hydrocortisone (HC) as the free alcohol and as 21-sodium succinate in concentrations of 1 and 3 \( \times 10^{-5} \) g/ml, respectively. (a) Strip contracted by epinephrine (E) and exposed to hydrocortisone before (left) and after (right) pretreatment with tropolone for 10 minutes. (b) Strip contracted by norepinephrine (NE) and exposed to hydrocortisone before (left) and after (right) pretreatment with U-0521 for 10 minutes. (c) Strip contracted by noradrenin (N) and exposed to hydrocortisone before (left) and after (right) pretreatment with U-0521 for 10 minutes. (d) Left, strip contracted by tyramine (TY) and exposed to hydrocortisone: right, strip from same aorta pretreated for 10 minutes with U-0521 and similarly exposed to tyramine and hydrocortisone. The potentiating effect of hydrocortisone and the 21-sodium succinate derivative on responses to norepinephrine was consistently abolished in both untreated and reserpine-pretreated strips after treatment with tropolone or U-0521. Similarly, responses to noradrenin and those of untreated strips to tyramine were not enhanced by hydrocortisone after inhibition of COMT (Fig. 4).

It was also observed that hydrocortisone enhanced the inhibitory responses of aortic strips of isoproterenol. This finding is in agreement with that of Besse and Base (6). To provide additional evidence that hydrocortisone and inhibitors of COMT have a common mechanism of action, the effect of U-0521 on potentiation by hydrocortisone of inhibitory responses to isoproterenol in aortic strips contracted by phenylephrine (1 \( \times 10^{-8} \) g/ml) was investigated. Preliminary responses to isoproterenol were determined in 46 strips from 10 reserpine-pretreated aortas. In most experiments, strips cut from the same aorta were used for all treatment groups to reduce variability, and their initial sensitivities to isoproterenol did not differ significantly. The strips were exposed to hydrocortisone (1 \( \times 10^{-5} \) g/ml) or to U-0521 (1 \( \times 10^{-5} \) g/ml) or to both hydrocortisone and U-0521. Ten minutes later, all the strips were recontracted by phenylephrine, and their responses to cumulative additions of isoproterenol determined. Maximal inhibition of phenylephrine-induced tone was produced by a concentration of 1 \( \times 10^{-7} \) g/ml.
isoproterenol (higher concentrations produced contractions). Responses to isoproterenol were potentiated about threefold by both hydrocortisone and U-0521. The effect of hydrocortisone plus U-0521 was no greater than that produced by U-0521 alone. These results are shown in Figure 6.

RELAXATION OF EPINEPHRINE CONTRACTED STRIPS IN OIL

Further evidence that hydrocortisone interfered with a major mechanism of catecholamine inactivation in aortic strips was obtained with the oil immersion technique. Epinephrine (1 × 10⁻⁸ g/ml) produced contractions in 17 strips of about half maximal amplitude, a mean of 23.7 ± 3.9 mm before oil immersion. Relaxation was 68.8 ± 2.2% complete in 5 minutes and 86.6 ± 1.2% complete in 15 minutes after oil immersion. After return to Krebs solution and recovery of basal tone, the strips were recontracted with the same concentration of epinephrine and exposed to hydrocortisone 21-sodium succinate (3 × 10⁻⁵ or 1 × 10⁻⁴ g/ml), or both concentrations in sequential tests, approximately 10 minutes before oil immersion. The results are summarized in Table 2, and traces from a typical experiment are shown in Figure 7a. Both concentrations of hydrocortisone 21-sodium succinate clearly decreased the rate at which aortic strips inactivated epinephrine. Relaxation was only

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**FIGURE 6**

Effects of U-0521 and hydrocortisone (HC) on responses of phenylephrine-contracted strips to cumulative concentrations of isoproterenol. Treatment conditions are described in text. Number of preparations represented by each curve is given in parentheses and standard errors of means are shown by vertical bars wherever they do not interfere with clarity.
Effects of hydrocortisone 21-sodium succinate (HC) on the relaxation of epinephrine-contracted aortic strips after oil immersion. (a) Strip contracted twice by epinephrine (E): left, control relaxation; right, strip exposed to hydrocortisone before oil immersion (O). (b) Strip contracted twice by epinephrine as above: left, strip pretreated with iproniazid and exposed to cocaine (C) before oil immersion; right, strip re-exposed to cocaine (effect of iproniazid is irreversible) and treated with hydrocortisone before oil immersion.

Table 2: Relaxation of Epinephrine-Contracted Aortic Strips in Oil

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. strips</th>
<th>Time to relax 50% (min)</th>
<th>Multiple of control time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17</td>
<td>2.9 ± 0.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Hydrocortisone (3 × 10⁻⁵)</td>
<td>17</td>
<td>5.2 ± 0.4</td>
<td>1.8</td>
</tr>
<tr>
<td>Hydrocortisone (1 × 10⁻⁴)</td>
<td>13</td>
<td>8.9 ± 0.7</td>
<td>3.0</td>
</tr>
<tr>
<td>Iproniazid plus cocaine (1 × 10⁻⁵)</td>
<td>15</td>
<td>5.5 ± 0.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Iproniazid plus cocaine (1 × 10⁻⁵) plus hydrocortisone (3 × 10⁻⁵)</td>
<td>11</td>
<td>13.7 ± 2.1</td>
<td>4.7</td>
</tr>
<tr>
<td>Iproniazid plus cocaine (1 × 10⁻⁵) plus hydrocortisone (1 × 10⁻⁴)</td>
<td>7</td>
<td>21.4 ± 2.4</td>
<td>7.3</td>
</tr>
</tbody>
</table>

*All drug concentrations are expressed in g/ml. The concentration of epinephrine was 1 × 10⁻⁶ g/ml. Hydrocortisone was used as the 21-sodium succinate.

†Values are means ± se.

53.6 ± 3.1 and 29.8 ± 3.1% complete in 5 minutes and 85.4 ± 1.3 and 73.8 ± 3.1% complete in 15 minutes after oil immersion in the lower and higher concentrations, respectively.

It was previously reported that the processes which inactivate epinephrine in aortic strips are enzymatic deamination and O-methylation and the uptake and storage of intact amine (18). The effect of hydrocortisone on the inactivation of epinephrine was also investigated in aortic strips in which MAO was inhibited with iproniazid and the uptake and storage processes with cocaine.

Fifteen strips from 8 aortas were pretreated with iproniazid, contracted with epinephrine (1 × 10⁻⁶ g/ml) for 10 to 20 minutes, and exposed to cocaine (1 × 10⁻⁵ g/ml) for an additional 10 minutes; their relaxation during 30 minutes of oil immersion
was then recorded. After return to Krebs solution and recovery of basal tone the strips were recontracted by epinephrine (the effect of iproniazid is irreversible), re-exposed to cocaine and, in addition, treated with hydrocortisone 21-sodium succinate \( (3 \times 10^{-6} \text{ or } 1 \times 10^{-4} \text{ g/ml}) \) 10 minutes before oil immersion. The records of a typical experiment are shown in Figure 7b and the data are summarized in Table 2. Hydrocortisone 21-sodium succinate markedly reduced the rate of relaxation of epinephrine-contracted aortic strips in oil after inhibition of MAO and uptake and storage processes. Strips relaxed 51.9 ± 2.9% in 5 minutes and 75.0 ± 2.5% in 15 minutes before, and 32.4 ± 4.3% and 20.8 ± 1.1% in 5 minutes and 59.6 ± 4.9% and 40.7 ± 5.4% in 15 minutes after treatment with hydrocortisone 21-sodium succinate in the lower and higher concentrations, respectively.

**Discussion**

Bohr and Cummings (8) and Fowler and Chou (9) had previously observed that responses of aortic strips to epinephrine and norepinephrine, respectively, were increased by hydrocortisone. These workers suggested that the adrenal steroids altered the transcellular potassium gradient in smooth muscle. Besse and Bass (6) studied the effects of hydrocortisone on the amplitude of response to a selection of sympathomimetic amines and reported that responses to synephrine and to catecholamines were enhanced. They found no evidence that hydrocortisone acted by altering the transmembrane potassium gradient and instead proposed that hydrocortisone acted at the level of the adrenergic receptors to increase their affinity for agonists with a \( p \)-hydroxy group.

In the present study, the only responses potentiated were to the amines that contained the catechol nucleus. Contractions in response to tyramine, which are mediated partly through the release of endogenous catecholamines (20, 21) were enhanced in untreated but not in reserpine-pretreated preparations. Since reserpine depletes tissues of catecholamines, it appears that the effect of norepinephrine released from tissue stores was enhanced by hydrocortisone.

The above findings suggested that hydrocortisone might specifically interfere with the inactivation of catecholamines in vascular tissue. This was supported by various experiments in which no difference was detectable between the effects of known inhibitors of COMT and hydrocortisone or hydrocortisone 21-sodium succinate.

It was previously reported that inhibition of COMT interfered with the inactivation of a low concentration of epinephrine much more than it did that of norepinephrine (18). This is due to the effectiveness of MAO as an alternate pathway for the inactivation of norepinephrine (18). In the present experiments, responses to epinephrine were increased much more than those to norepinephrine by known COMT inhibitors and by hydrocortisone, which provided further evidence of a common mechanism of action.

The intravenous administration of hydrocortisone enhances the pressor effects of epinephrine but not norepinephrine in the cat and rabbit (5) and dog (6) and the constrictor response to epinephrine in the de-nervated pump-perfused hindquarters of cats (7). These reports can also be explained by the observation that MAO is much more effective as an alternate pathway of norepinephrine than epinephrine metabolism. In addition, it is known that more epinephrine than norepinephrine is O-methylated during the first 5 minutes after the injection of tritiated amine (22, 23). In the present experiments hydrocortisone was added to the muscle chambers after contractions in response to norepinephrine had reached a plateau and the very slight increase in response amplitude was readily observable.

The most definitive evidence that hydrocortisone effectively decreased the rate of O-methylation of catecholamines under the present experimental conditions was obtained with the oil immersion technique. Maximum inhibition of COMT with tropo-
Hydrocortisone potentiation

Lone increased the time for aortic strips to relax 50% to 2.9 times that of the controls (18), an effect similar to that observed with hydrocortisone 21-sodium succinate (1 x 10^-4 g/ml) in the present experiments.

Hydrocortisone 21-sodium succinate also decreased the rate of relaxation of epinephrine (1 x 10^-8 g/ml) contracted strips in oil after inhibition of MAO and uptake and storage processes. Exposure of such pretreated strips to the COMT inhibitor, tropolone, almost completely eliminated their residual capacity to inactivate epinephrine (18). This finding demonstrated that no other independent mechanism of consequence operates to inactivate epinephrine in aortic strips. Exposure of strips treated with iproniazid and cocaine to 3 x 10^-6 and 1 x 10^-4 g/ml hydrocortisone 21-sodium succinate reduced their residual inactivation capacity by 60% and 74%, respectively. This effect is only slightly less than that produced by maximal inhibition of COMT by tropolone, a reduction of 92%.

The higher concentration of hydrocortisone 21-sodium succinate produced a greater effect than the lower on the inactivation of epinephrine, although the higher concentration did not produce a materially greater potentiation. This finding would be explained if vascular tissue had the capacity to inactivate some of the hydrocortisone trapped in the tissue during oil immersion. Thomas et al. (24) and Travis and Sayers (25) have reported that strips of skeletal muscle and the heart-lung preparation, respectively, can metabolize hydrocortisone. Evidence from the present experiments supporting such a possibility for vascular tissue was the finding that the lower concentration of hydrocortisone produced its greatest effect on relaxation 5 minutes after oil immersion. By 15 minutes, relaxation had returned toward that of control strips.

The sum of the evidence presented above clearly demonstrates that hydrocortisone, in a concentration no greater than that required of known inhibitors, markedly decreases the rate at which catecholamines are O-methylated in vascular tissue; thus explaining the enhancement of responses to catecholamines. Future work must determine whether or not COMT is effectively inhibited by hydrocortisone in all tissues which contain the enzyme. It has recently been shown that certain agents decrease the rate at which tissue metabolize catecholamines by inhibiting their access to sites of enzymatic degradation rather than by direct inhibition (26, 27). The possibility must be considered that hydrocortisone also simulates enzyme inhibition, by blocking the movement of catecholamines across membranes, to sites of COMT activity.

Ramey and Goldstein (28) proposed that in the absence of the adrenal cortical steroids the threshold for all reactions in which epinephrine and norepinephrine participate is increased, although the validity of this hypothesis has been recently challenged (29-31). It is unlikely that there is a relationship between impaired responses to catecholamines in adrenalectomized animals, which probably involve a multiplicity of factors, and the present finding that hydrocortisone is an inhibitor of COMT. However, the concentrations of adrenal cortical steroids present in tissues and tissue fluids, especially during prolonged hypercorticism, could exert some degree of control over the rate at which catecholamines are O-methylated. The concentration of hydrocortisone in peripheral plasma of normal man has been estimated at 0.1 /µg/ml (32), which is much less than that required for maximal enhancement of responses to epinephrine and norepinephrine in the present experiments (10 /µg/ml), but peripheral tissues and nerves of the cat, in vivo, have been shown to concentrate hydrocortisone to many times the levels in plasma (33).

There have been reports that corticosterone, aldosterone and a number of other steroid hormones enhance the sensitivity of smooth muscle to catecholamines (e.g. 8, 9). It would not be surprising if these compounds also produce their effects by inter-
ferring with the inactivation of catecholamines.

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References


Mechanism of Hydrocortisone Potentiation of Responses to Epinephrine and Norepinephrine in Rabbit Aorta

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