Interactions of Mineralocorticoids and Pressor Agents in Vascular Smooth Muscle

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SUMMARY The direct effects of aldosterone and other mineralocorticoids on vascular smooth muscle were evaluated in isolated rabbit ear artery and aorta preparations. In the ear artery, aldosterone alone produced only a minimal effect. However, if desipramine, a blocker of neuronal norepinephrine uptake, was first placed in the tissue bath, aldosterone (10^-6, 10^-5, and 10^-4 M, n = 10 in each study) gave contractile responses of 0.2 ± 0.05 (SE), 0.5 ± 0.1, and 1.4 ± 0.2 g. In tissues in which neuronal norepinephrine stores were depleted by cold-storage, desipramine and aldosterone (10^-4 M) produced a contraction of only 0.12 ± 0.06 g, but if the norepinephrine stores were replenished, the contractions averaged 1.2 ± 0.3 g. The effects of aldosterone could be abolished by phentolamine, again suggesting that this effect was adrenergically mediated. The aorta studies utilized an oil immersion technique that prevents diffusion of active substances from receptor compartments and allows evaluation of postcontractile relaxation. Following pretreatment with norepinephrine and desipramine, the relaxation half-times (time for 50% relaxation) after aldosterone 10^-6, 10^-5, and 10^-4 M averaged 7, 12, and 18 minutes. Contractions produced by deoxycorticosterone, spironolactone, or aldosterone, given singly or in combination, were closely similar to each other, and their relaxation half-times were greater than control (18 vs 7 minutes). Aldosterone produced similar effects if epinephrine was substituted for norepinephrine in the bath; with angiotensin II, however, the contractile response to aldosterone was less, although the relaxation half-time was significantly prolonged. It is suggested that aldosterone and other mineralocorticoids might contribute to vascular tone by enhancing and prolonging the contractile effectiveness of other pressor substances. It is likely that this effect is not mediated by a classical mineralocorticoid action, but instead may be dependent upon the inhibition of the extraneuronal uptake of catecholamines.

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KEY WORDS • mineralocorticoids • aldosterone • vasoconstriction • catecholamines • angiotensin II • rabbit

I t is well established that the mineralocorticoid hormone, aldosterone, is able to influence systemic blood pressure in humans.1 The most widely recognized action of aldosterone is in the distal renal tubule where it enhances reabsorption of sodium and the secretion of potassium. During studies of clinical hypertension, however, it has been speculated that aldosterone may contribute to elevated blood pressure levels by mechanisms additional to its effects on fluid and electrolyte balance.2 In particular, it is possible that adrenocortical hormones such as aldosterone may have effects on the circulation through direct actions on vascular tissue. Studies carried out principally in in vitro preparations have shown that corticosteroids can enhance the vasoconstrictor actions of administered catecholamines.3,4

In this study, we examined the effectiveness of mineralocorticoid hormones in enhancing the contractile actions of catecholamines and other pressor substances. We have utilized two separate types of in vitro vascular preparations obtained from the rabbit: 1) the ear artery, in which vascular tone is sustained by endogenous catecholamines even during prolonged in vitro studies; and 2) the aorta, a preparation in which the differing effects of exogenously administered pressor substances can be compared. Our results indicate that aldosterone and its related hormones can prolong as well as increase the amplitude of the constrictor effects of the catecholamines, and that this action is due principally to an ability of the mineralocorticoids to inhibit extraneuronal uptake of the catecholamines.
Methods

The studies were performed in New Zealand white rabbits, 2.5 to 3 kg, fed with a standard laboratory diet. Before each experiment, the animals were stunned and exsanguinated, and the central ear artery or thoracic aorta dissected out. The vessels were carefully separated from their surrounding connective tissue and divided into cylindrical segments approximately 3 mm in length. Throughout these procedures, the vascular tissue was immersed in Krebs-bicarbonate solution gassed with a 95%/5% mixture of O₂/CO₂. The physiological solution was made up of sodium chloride (119.2 mM), sodium bicarbonate (25 mM), potassium chloride (4.9 mM), calcium chloride (1.3 mM), magnesium sulfate (1.2 mM), glucose (11.1 mM), ascorbic acid (0.114 mM), and EDTA (0.03 mM). The vascular segments were then mounted in smooth muscle tissue baths as described by Bevan and Osher. The bath consisted of a glass chamber enclosed by hollow walls through which heated water was pumped to maintain a constant temperature of 37°C. The interior chamber, containing the tissue and bathing medium, continuously received the O₂/CO₂ mixture which oxygenated the tissue, provided the carbonic acid component of the bicarbonate-buffered Krebs solution, and insured mixing of the medium. The arterial segment was suspended on two stainless steel wires passed through the lumen: the lower of the wires was attached to a rigid support, whereas the uppermost wire was linked by fine nylon threads to a Grass force-displacement transducer (FT03C) mounted above the bath and linked to a Grass polygraph recorder (Model 7).

The tissues were equilibrated for 30 minutes under a previously determined optimal resting force of 1.5 g. They were then exposed to norepinephrine in a final bath concentration of 10⁻⁷ M. The vessels were allowed to contract until a steady state response occurred, after which the baths were drained and refilled with fresh Krebs solution. The norepinephrine exposure was repeated until each tissue gave uniform responses. Tissues not contracting to a minimum of 3 g were discarded as being insufficiently reactive. After the last exposure to norepinephrine, a further 20 minute recovery period was allowed before experimental exposure to other agents. In general (for exceptions see later), desipramine (1 × 10⁻⁷ M) was then added to the tissue baths. This concentration of desipramine was chosen because, at low substrate concentrations, it has been shown to provide near maximal blockade of the neuronal (presynaptic) uptake while exerting little or no effect on post-synaptic tissues. Functional evidence that 1 × 10⁻⁷ M desipramine blocks neuronal uptake in the rabbit ear artery has also been obtained. In the ear artery preparations, differing concentrations of aldosterone (see Results) were then given. In a further set of studies, the alpha-adrenergic agent, phentolamine, was administered either before the aldosterone or after the aldosterone had achieved its maximum effects. An additional series of ear artery tissues were removed from the animal as described above, but were then placed in Krebs-bicarbonate solution and stored in the cold (2°C) for two days as described in the method of Shibata et al. for producing depletion of endogenous catecholamines. Following this procedure, the tissues were placed in Krebs solution at 37°C as described above. However, these tissues were exposed to methoxamine 10⁻⁷ M rather than to norepinephrine for the preexperimental contractile testing; although methoxamine has a powerful post-synaptic stimulatory action, it is not a substrate for neuronal (presynaptic) uptake and thus does not deplete the labile neuronal catecholamine stores depleted by the exposure to cold. Prior to the final pre-experimental washing, however, 16 of these preparations were exposed to norepinephrine 10⁻⁷ M, whereas nine tissues received a final administration of methoxamine.

The aorta segments were studied according to the following modification of the oil immersion technique of Kalsner and Nickerson. Thirty minutes after addition of the desipramine, norepinephrine (or another vasoconstrictor agent) was added cumulatively in geometric progression until a steady-state contraction of 1.5–3.5 g was achieved. This was approximately 20% to 45% of the maximal contractile response obtained with norepinephrine. Soon after the steady-state response was achieved, either aldosterone, spironolactone, deoxycorticosterone acetate (DOCA), or the combination of DOCA with either aldosterone or spironolactone was added to the baths, and the tissues were allowed to contract further. Upon reaching the second steady-state response, the baths were drained and refilled with mineral oil warmed to 30°C and gassed with O₂/CO₂ (95%/5%). The rate and extent of tissue relaxation was then recorded.

The steroid compounds, obtained from Sigma Chemical Company (St. Louis, Missouri), were all made up in ethanol; the desipramine (USV Pharmaceutical Corporation, Tuckahoe, New York) and angiotensin II (Ciba Pharmaceutical Company, Summit, New Jersey) were made up in distilled water, and all other agents were made up in 0.001 M hydrochloric acid. Other compounds were methoxamine (Burroughs Wellcome, Research Triangle Park, North Carolina), norepinephrine and epinephrine (Sigma Chemical Company, St. Louis, Missouri), and light mineral oil (Mallinkrodt, Los Angeles, California). Statistical analysis was carried out using unpaired one-tailed t tests. Values are given as mean ± standard error of the mean.

Results

Ear Artery Experiments

In 16 ear artery preparations, addition of aldosterone alone (10⁻⁴ M) produced a mean contraction of 0.07 ± 0.03 g; when desipramine, 10⁻⁷ M, was added to the aldosterone, the contraction increased to 0.8 ± 0.07 g. In 16 additional tissue preparations, desipramine alone produced a contraction of 0.22 ± 0.05 g; the addition of aldosterone then increased the contraction to 1.52
± 0.22 g. The final contraction produced in the second set of tissues was significantly greater ($p < 0.001$) than that produced in the first set of tissues; thus, aldosterone produced its greatest contraction when added to tissues already pretreated with desipramine. Three further sets of tissues ($n = 10$ in each case) were also pretreated with desipramine $10^{-7}$ M; the addition of aldosterone ($10^{-6}$ M) produced a contraction of $0.18 \pm 0.04$ g; aldosterone ($10^{-5}$ M) produced a contraction of $0.48 \pm 0.06$ g; and aldosterone ($10^{-4}$ M) produced a contraction of $1.38 \pm 0.16$ g. The response to aldosterone ($10^{-5}$ M) was greater ($p < 0.05$) than to aldosterone ($10^{-6}$ M), and that to aldosterone ($10^{-4}$ M) was greater ($p < 0.01$) than that to aldosterone ($10^{-3}$ M). In the tissues treated with desipramine and aldosterone ($10^{-4}$ M), the addition of phentolamine ($10^{-7}$ M) completely abolished the contractile effect. In 10 separate tissues, phentolamine was administered immediately after desipramine and produced a small ($0.26 \pm 0.04$ g) but significant ($p < 0.02$) decrease in the resting contractile tone. If aldosterone ($10^{-4}$ M) was then added, there was no further change in the contractile state of the tissue. Thus, phentolamine could both abolish and prevent the contractile effects of aldosterone in the rabbit ear artery preparation.

The effects of administering desipramine and aldosterone to tissues subjected to the catecholamine-depleting technique of Shibata et al. are shown in figure 1. In tissues in which methoxamine was used instead of norepinephrine for the final preexperimental viability testing, there was only a minimal response to the desipramine and aldosterone. However, if norepinephrine was restored to the tissues prior to the experiment, the typical contractile effects of the desipramine and aldosterone occurred. Thus, in all 16 tissues pretreated with the norepinephrine and desipramine, aldosterone increased the contractile state (average increment: $0.48 \pm 0.17$ g, $p < 0.01$); this effect was significantly greater ($p < 0.01$) than when the aldosterone was added to the nine tissues pretreated with methoxamine and desipramine. In this latter group, two separate patterns were observed. In the six preparations in which desipramine produced contractions of 1.0 g or more, the desipramine-induced contractions exceeded those produced by aldosterone by an average of $0.8 \pm 0.4$ g. In the remaining 10 preparations in which the opposite pattern occurred, the force of the aldosterone contractions exceeded those of desipramine by an average of $0.8 \pm 0.2$ g. The difference between these diverging trends was significant: $t = 3.52, p < 0.01$.

The data are shown in figure 2.

**Aorta Studies**

In preparations in which the tissue bath fluid was substituted by mineral oil following addition of the active test substances, it was possible to measure the time course of the relaxation of the contractile responses to the steroid substances. In these studies, the tissues were pretreated with norepinephrine and then with desipramine; the effects of aldosterone (and other corticosteroids) were then superimposed upon the steady-state contractions produced by the pretreatment. Figure 3 shows the relative relaxation rates of aorta tissues treated with aldosterone in concentrations of $10^{-6}$ M, $10^{-5}$ M, or $10^{-4}$ M. As with the absolute height of the contractile response, the rate of relaxation appeared to be concentration-dependent, being slowest with the high concentration of aldosterone and most rapid in the presence of a low concentration. Thus, aldosterone appeared to prolong as well as increase the contractile response. Experiences with aldosterone, spironolactone, deoxycorticosterone acetate (DOCA), and combinations of these substances, are summarized.
Aldosterone

\[ 1 \times 10^{-5} \text{M} \]
\[ 1 \times 10^{-4} \text{M} \]
\[ 1 \times 10^{-3} \text{M} \]

**FIGURE 3.** Average relaxation values \((n = 8\) for each concentration) in rabbit aorta tissues treated with aldosterone (for technique, see text).

\[ C = \text{Control} \]
\[ A = \text{Aldosterone} \]

**FIGURE 4.** Relaxation half-times following administrations of ethanol alone (control) or aldosterone \((10^{-4} \text{M})\) in rabbit aorta tissues pretreated with norepinephrine \((n = 7)\), epinephrine \((n = 13)\), or angiotensin II \((n = 4)\). Values are mean \(\pm\) SEM.

**TABLE 1.** Contractile Effects and Relaxation Half-Times \((t/2)\) of Steroid-Treated Rabbit Aorta Segments During Mineral Oil Immersion

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Contraction</th>
<th>Relaxation (t/2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol (control) alone</td>
<td>50.7 (\pm) 4.1</td>
<td>39.0 (\pm) 5.0</td>
</tr>
<tr>
<td>Aldosterone ((1 \times 10^{-4} \text{M}))</td>
<td>92.7 (\pm) 7.4</td>
<td>99.4 (\pm) 17.4</td>
</tr>
<tr>
<td>Aldosterone + DOCA</td>
<td>94.5 (\pm) 8.0</td>
<td>97.8 (\pm) 11.8</td>
</tr>
<tr>
<td>Spironolactone ((1 \times 10^{-4} \text{M}))</td>
<td>99.5 (\pm) 10.1</td>
<td>103.4 (\pm) 10.1</td>
</tr>
<tr>
<td>Spironolactone + DOCA</td>
<td>91.8 (\pm) 9.3</td>
<td>95.5 (\pm) 7.9</td>
</tr>
</tbody>
</table>

Values are expressed as percentage of effect of DOCA \((3 \times 10^{-3} \text{M})\) alone (Contraction average: 1.63 \(\pm\) 0.13 g/60 min; average: 17.8 \(\pm\) 1.0 g/60 min) and are given as mean \(\pm\) SEM, \(n = 16\). All treatment values differ significantly \((p < 0.05)\) from those during ethanol (control) alone.

Discussion

In studies performed in the rabbit ear artery we found that aldosterone produced a concentration-dependent increase in vascular contractility. Although it is difficult to draw physiologic conclusions from this type of study, the data are consistent with the possibility that aldosterone (and related substances) might participate in circulatory regulation by direct action within arterial tissue. We found that this action of aldosterone required the prior administration of desipramine which is a blocker of neuronal catecholamine uptake. Thus, it seems likely that the constrictor effects of aldosterone in the ear artery preparation are dependent upon the availability of threshold amounts of the neurotransmitter, norepinephrine.

The importance of endogenous norepinephrine in the effects of aldosterone was emphasized by the studies in which neuronal catecholamine stores were depleted by the cold-storage technique. In these studies,
methoxamine, which is not a substrate for neuronal catecholamine uptake, was used instead of norepinephrine for the pre-experimental viability testing of the tissues. We found that desipramine and aldosterone produced only minimal contractile responses under these circumstances. However, if the labile neuronal catecholamine stores were repleted by the addition of norepinephrine, desipramine and aldosterone now produced the customary powerful vasoconstriction. Two separate patterns of response were observed in these latter studies. In 6 of the 16 experiments (fig. 2), desipramine by itself produced large contractile responses; when this occurred, the subsequent additional contraction observed when aldosterone was administered tended to be relatively small. In contrast, when desipramine produced only small contractile responses, the addition of aldosterone then tended to stimulate substantial contractile increments. This observation supports the idea suggested previously by Graefe and Trendelenburg that adrenocortical steroids may produce their most important enhancement of vasoconstriction in conditions where resting sympathetic drive is relatively low. It is not entirely clear why we observed the two types of responses in these in vitro studies, but it is possible that changes in nerve terminal mechanisms produced by the cold storage procedure might have predisposed to an increased sensitivity to the effects of desipramine in some of the preparations.

The mechanism by which aldosterone increased the contractile effectiveness of the norepinephrine is not certain, but probably is best explained by an inhibitory effect on extraneuronal catecholamine uptake (uptake-2). It has been shown previously that corticosteroids, including the aldosterone precursor, deoxycorticosterone, are able to decrease extraneuronal norepinephrine uptake. A decrease in the extraneuronal uptake of tritiated-norepinephrine by aldosterone has also been reported. Thus, it could be postulated that the contractile effects of aldosterone are dependent upon its ability to retard the uptake and metabolism of catecholamines at tissue sites, thereby enhancing the effectiveness of these pressor substances. In support of this idea was our finding that the contractile effects of aldosterone could be completely abolished by the addition of the alpha-adrenergic blocking agent, phentolamine, to the tissue bath. Similarly, the presence of phentolamine in the tissue bath prior to the administration of desipramine and aldosterone totally prevented any contractile responses. Since there is no evidence to suggest that aldosterone has direct actions at alpha-adrenergic receptors, it seems highly likely that its effects in the rabbit ear artery were mediated through norepinephrine.

Under normal circumstances, norepinephrine is removed from the receptor compartment of vascular smooth muscle by neuronal uptake, extraneuronal uptake and diffusion. Thus, to test the importance of the postulated extraneuronal mechanism in our studies with adrenocortical hormones in the rabbit aorta preparations, we blocked neuronal uptake of norepinephrine with desipramine, and prevented diffusion by substituting mineral oil for the tissue bath fluid following addition of the active substances. It has been shown previously that removal of catecholamine from the receptor compartment under these conditions is due primarily to the activity of the extraneuronal-uptake-COMT system. Moreover, the activity of this catecholamine-removal system appears to be reflected closely by changes in the contractile state of the tissue. Using this method, we found that aldosterone produced a concentration-dependent effect on the rate of relaxation of the vascular tissue. Thus, high concentrations of aldosterone not only produced greater amplitudes of contractile response, but also sustained these effects for longer than was found with the lower concentrations.

In further aorta ring studies, we compared the relative effects of three mineralocorticoid substances: deoxycorticosteroid acetate (DOCA), aldosterone, and spironolactone. When compared with the effects of ethanol, which was the diluent for the other substances and was thus used as a control, the steroids all produced significantly greater amplitudes of contraction and prolonged the relaxation half-time (time for the contractile response to decline by 50%) at least two-fold. There were no differences between the three steroids in these effects; moreover, when aldosterone or spironolactone were given in combination with DOCA, the responses were not different from those elicited by the steroids given individually. Thus, their effects on vascular contractility were probably mediated by the same mechanism, in all likelihood the blockade of extraneuronal norepinephrine uptake (as discussed above).

Spironolactone is known to inhibit the intrarenal effects of aldosterone on sodium and potassium exchange. In the present study, however, spironolactone appeared to exhibit contractile properties identical to those of aldosterone. This suggests that the phenomena observed in this study are probably not dependent upon changes in electrolyte flux, although this possibility cannot be excluded. Indeed, it has been suggested previously that corticosteroids are able to influence the movement of sodium, potassium, and calcium across smooth muscle membranes and thereby alter sensitivity to vasoconstrictor stimuli. In the present study, however, the failure of aldosterone to produce substantial contractile effects in the absence of desipramine suggests that the availability of neurotransmitter was the predominant factor in aldosterone's action.

In further studies with the oil immersion technique we found that there was little change in the eventual response when epinephrine was substituted for norepinephrine as the pressor agent in the aorta preparations. It was also observed that the pressor response to angiotensin II could be prolonged by aldosterone in a fashion similar to that observed for norepinephrine and epinephrine. The explanation for this finding is not clear, for it is not established whether aldosterone can influence the rate of removal of angiotensin II from its receptor sites. But it is possible to speculate that this angiotensin-aldosterone interaction also might be me-
iated through catecholamine mechanisms. It has been shown that angiotensin enhances the pressor effectiveness of norepinephrine by stimulating its release from autonomic nerve terminals, and perhaps also by interfering with its reuptake. It is thus possible that aldosterone increases the contractile effects of angiotensin II by enhancing that component of angiotensin’s action that is dependent upon its interrelationships with endogenous norepinephrine mechanisms.

Interactions between adrenocortical hormones and catecholamines have been established in the past. Studies have shown that the presence of intact adrenal glands or of exogenous adrenocortical hormones is necessary for norepinephrine to exhibit its full pressor effects. Moreover, in a variety of vascular preparations, the vasoconstrictor effects of norepinephrine and epinephrine have been shown to be potentiated by administration of hydrocortisone or cortisone. Corticosteroids also may directly influence circulating hemodynamics through changes in the central nervous system or at the carotid sinus. These latter findings, however, do not help explain our observations in the in vitro preparations used in the present study.

The enhancement and prolongation of the effects of pressor substances (chiefly catecholamines) by mineralocorticoids observed in this study are yet to be placed in a physiological context. The tissue bath concentrations of aldosterone used in this investigation are high when compared with those in human plasma, although it has been speculated that the complex binding characteristics of aldosterone might concentrate it to a high degree in the vicinity of its receptors. Moreover, it has been shown that neuronal tissue concentrations of adrenocortical steroids may be several hundredfold higher than in plasma. Caution must still be employed, however, in determining how the findings in this report can be used to explain physiologic or clinical phenomena.

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