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A Fast-Acting Humoral Factor Mediates Pressor Hyperresponsiveness in Deoxycorticosterone-Salt Rabbits (42011)

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Abstract. Six rabbits were sham operated and were given water to drink (sham-water group); six additional rabbits were sham operated and were given saline to drink (sham-salt group); another six rabbits received an implant of deoxycorticosterone (DOCA) and were given water to drink (DOCA-water group); a final group of six rabbits received implants of DOCA and were given saline to drink (DOCA-salt group). Two weeks later, all four groups of rabbits had approximately the same mean arterial pressures, and the sham-salt, DOCA-water, and DOCA-salt groups all had plasma renin activity values less than the sham-water group. The DOCA-salt group had greater pressor responses to norepinephrine (NE) at several doses than did the other three groups of rabbits. In another group of six sham-water and six DOCA-salt rabbits, measurements of cardiac output before and during infusions of NE at 800 ng/min/kg body wt revealed no changes in cardiac output before or during NE infusion, but the DOCA-salt group had significantly greater increases in mean arterial pressure and total peripheral resistance during NE than did the sham-water group. In another group of six DOCA-salt rabbits, the pressor response to several doses of NE were determined during infusion of the angiotensin II (AII) antagonist, [Sar^1, Ile^8] AII; this AII antagonist failed to alter the enhanced pressor responses to NE. A final experiment examined pressor responses to NE in six normal rabbits before and after cross circulation of blood with six DOCA-salt rabbits. After blood cross circulation the normal rabbits had exaggerated pressor responses to NE at 5, 15, and 30 min, but not at 60 min. Similar cross-circulation experiments between six pairs of normal rabbits did not show any transfer of pressor hyperresponsiveness. These studies indicated that pressor and vascular hyperresponsiveness in DOCA-salt rabbits is conveyed by a fast-acting hormonal factor and that AII probably is not involved in mediating this hyperresponsiveness.

Earlier studies from this laboratory have demonstrated that one-kidney rabbits with renal artery stenosis (one-kidney, one-clip) (1) and two-kidney rabbits with unilateral renal artery stenosis (two-kidney, one-clip) (2) have exaggerated increases in mean arterial pressure in response to infusions of norepinephrine (NE) prior to the development of hypertension, and infusion of the angiotensin II (AII) antagonist, [Sar^1, Ile^8] AII, blocks this pressor hyperresponsiveness in both of these models. The present study examined the pressor and vascular responses to NE in rabbits after the application of another forcing known to produce hypertension in animals, i.e., the administration of deoxycorticosterone acetate (DOCA) and a high salt intake. This study also examined the effects of [Sar^1, Ile^8] AII on the pressor responses to NE in DOCA-salt rabbits. Lastly, this study investigated the possibility that a fast-acting humoral factor may be involved in mediating pressor hyperresponsiveness in DOCA-salt rabbits.

Methods. A total of 66 male New Zealand white rabbits, ranging 2.6 to 3.5 kg in body weight, were used in these studies. The rabbits were housed one per cage and were kept in a constant environmental temperature of 26°C. Room lights were controlled by an automatic time switch that provided illumination from 7:00 AM to 7:00 PM daily. All rabbits received an excess of a commercial rabbit diet (Purina Lab Rabbit Chow

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HF5326) containing 165 meq of Na^+ and 457 meq of K^+/kg of feed. For drinking, rabbits received an excess of either tap water or a salt solution containing 9 g of NaCl plus 2 g of KCl/liter of water.

Surgical procedures. Prior to surgery each rabbit was weighed to within 0.01 kg. All rabbits were then anesthetized with halothane plus nitrous oxide in oxygen (3). With sterile surgical procedures, the rabbits designated as DOCA treated had a pellet of DOCA implanted subcutaneously in the dorsal region between the scapulae; the DOCA pellet contained 250 mg of pure material and was in the form of a firm disc 13 mm in diameter and 2 mm thick. Rabbits designated as sham operated were anesthetized and the incision was made, but the DOCA pellet was not implanted. All rabbits were closed and were returned to their cages after surgery. Some rabbits used in acute experiments (Experiment 4) were not subjected to surgery prior to the day of the acute experiment.

On the day of an acute experiment each rabbit again was anesthetized with halothane plus nitrous oxide. Polyvinyl catheters (Fr 5 infant feeding tubes) were placed in the lower aorta and inferior vena cava via the femoral artery and vein. Rabbits in which cardiac output measurements were to be obtained (Experiment 2) also had a similar catheter inserted into the jugular vein and placed so that the catheter tip would lie near the right atrium. Following these catheter placements, each rabbit was placed in a rectangular box to restrict its movements. Each rabbit was unrestrained and was restricted only by the size of the box. The rabbit remained in the box until the conclusion of the experiment. Acute experiments were performed on conscious rabbits 6 hr after implanting the catheters. A total of four experiments were performed.

Experiment 1. Four groups of six rabbits each were used in these experiments. The first group consisted of sham-operated rabbits given water to drink (sham-water group). The second group of rabbits also was sham operated but was given the salt solution to drink (sham-salt group). The third group was treated with DOCA and was given water to drink (DOCA-water group). The fourth group was DOCA treated and received the salt drinking solution (DOCA-salt group). These treatments were continued for 14 days, and at that time the acute experiment was performed. Six hours after the vessel catheters were placed, each rabbit was weighed, and an arterial blood sample (2.5 ml) was obtained. Approximately 1.5 ml of the blood was added to a chilled tube containing EDTA, for the determination of plasma renin activity (PRA); the remainder was placed in a heparin tube for the determination of plasma concentrations of Na^+ and K^+. The arterial catheter was attached to a pressure transducer, and control measurements of mean arterial pressure were recorded. Solutions of NE were infused iv at 25, 50, 100, 200, 400, 800, and 1200 ng/min/kg of body wt, and the pressor response to each dose was recorded. The NE solutions were prepared by diluting a commercial NE preparation (Levophed, Breon Laboratories) with 5% dextrose in water. The increase in mean arterial pressure by the fifth minute of NE infusion was taken as the pressor response. An interval of at least 5 min was allowed between infusions; the next dose of NE was not infused until the mean arterial pressure had returned to the control level and had stabilized.

Experiment 2. Two groups of six rabbits each were used in these experiments. The first group of rabbits was sham operated and was given water to drink. The second group of rabbits was implanted with DOCA and was given the salt solution to drink. Fourteen days later, the acute experiment was performed. On the day of an acute experiment, 6 hr after placement of the vessel catheters, control measurements were obtained for mean arterial pressure and cardiac output. Measurements of cardiac output were by dye dilution with indocyanine green dye (Cardio-Green; Hynson, Westcott, and Dunning, Inc.). The technique used in this laboratory for measuring cardiac output by this method has been described previously (4). Each cardiac output determination was made in triplicate, and the average of the three determinations was accepted as the measured value. Following these control measurements, a solution of NE was infused iv at 800 ng/min/kg of body wt, and the pressor response was

recorded. During the fifth minute of NE infusion, measurements again were obtained for cardiac output.

Experiment 3. A group of six rabbits was implanted with DOCA and was given the salt solution to drink. Fourteen days later the acute experiment was performed. After control measurements of mean arterial pressure were obtained, NE was infused iv at 800 ng/min/kg of body wt, and the pressor response was recorded. The NE infusion was then stopped. After mean arterial pressure had returned to normal levels, an injection of 300 ng of synthetic AII was made into each rabbit, and the pressor response was recorded. Each rabbit then received an iv infusion of [Sar^1, Ile^8] AII in saline, at 300 ng/min/kg of body wt. After 30 min of infusion of this AII antagonist, the pressor response to an iv injection of 300 ng of AII again was determined. While infusion of the AII antagonist was continued, the pressor responses to infusions of NE at 25, 50, 100, 200, 400, 800, and 1200 ng/min/kg of body wt were obtained, as in Experiment 1. After infusion of the last dose of NE, the pressor response to 300 ng of AII again was obtained.

Experiment 4. This experiment required 6 DOCA-salt rabbits and 18 rabbits that were unoperated prior to the acute experiments. These experiments were performed on rabbits in pairs, with each pair consisting of a donor and a recipient rabbit. In the first phase of this experiment the donor rabbit of each pair was a DOCA-salt rabbit and the recipient was a normal rabbit. In the second phase, both the donor and recipient rabbits were normal. Each rabbit was given an iv injection of 1000 units of heparin. Mean arterial pressure was measured, and the pressor response to an iv infusion of NE at 800 ng/min/kg body wt was recorded in each rabbit of the pair. The NE solution was then removed from the venous catheters. The femoral catheters of the donor and recipient rabbit of each pair were then connected to the tubing of a single-roller, dual-chamber infusion pump in a manner such that in one chamber the blood would be pumped from the femoral artery of the donor rabbit into the femoral vein of the recipient, and in the other chamber the blood would be pumped from the femoral artery of the recipient rabbit into the femoral vein of the donor. The pump tubing had a volume of approximately 3 ml and was filled with isotonic saline prior to being connected to the rabbit catheters. At the start of the cross circulation the pump speed was adjusted so that the rate of blood exchange between the pairs of rabbits would be 10.0 ml/min. Because both pump chambers were connected to the same roller, the rate of exchange of blood between the two rabbits in each pair was the same. Blood cross circulation was continued for 5 min; the pump was then stopped, and the pressure transducer was connected to the femoral arterial catheter of the recipient rabbit. Pressor responses to NE infused again at 800 ng/min/kg body wt were determined in the recipient rabbits at 5, 15, 30, and 60 min after the termination of the cross circulation.

Analytical procedures. Values for PRA were obtained by radioimmunoassay of generated angiotensin I, by a modification of the method of Cohen *et al.* (5). This modification, as used in our laboratory, has been described in detail previously (4). Values for PRA are expressed as nanogram of angiotensin I generated per hour of incubation, per milliliter of plasma. Plasma concentrations of Na^+ and K^+ were determined by flame photometry.

Statistics. Values for body weight, mean arterial pressure, PRA, plasma concentrations of Na^+ and K^+, and the pressor response to each dose of NE were compared among the four groups of rabbits in Experiment 1 by analysis of variance; when significant values were found ($P < 0.05$), the data were analyzed further by Duncan's new multiple range test (6). Changes in body weight that occurred during the treatment period in Experiment 1 were tested by the u test (6) to determine if these changes were significantly different from 0. The initial cardiovascular values, as well as the changes in the cardiovascular values during NE infusion, between the two groups of rabbits in Experiment 2 were tested by Student's t test for group observations (6). The effect of NE infusion on the cardiovascular factors for each group of rabbits in this experiment was tested by Student's t test for paired observations (6). Values for mean arterial pressure and the pressor responses to

each dose of NE were compared between the rabbits in Experiment 3 and the DOCA-salt rabbits in Experiment 1 by Student's t test for group observations. The pressor responses to NE at 800 ng/min/kg before and during infusion of [Sar^1, Ile^8] AII in the rabbits in Experiment 3 were compared by Student's t test for paired observations. In Experiment 4 the values for body weight, mean arterial pressure, and the pressor responses to NE were compared between the donor and recipient rabbits in each phase by Student's t test for group observations. After blood cross circulation the pressor responses to NE at each time period in the recipient rabbits were compared to the pressor responses in these rabbits prior to cross circulation by the u test, to determine if the differences in the pressor responses were significantly different from 0. In these studies, significance levels were accepted as $P < 0.05$ and $P < 0.01$.

Results. Table I summarizes the values for initial and final body weights, mean arterial pressure, PRA, and plasma concentrations of Na^+ and K^+ for the four groups of rabbits in this experiment. Control values for initial body weight and mean arterial pressure were not different among the four groups. The sham-water group had a significant ($P < 0.05$) gain in body weight by an average of 3% during the 14-day treatment period, while the DOCA-salt group had a significant ($P < 0.05$) loss of body weight by an average of 9% during this period; the sham-salt and DOCA-water groups had no significant changes in body weights. Values for PRA in the sham-salt, DOCA-water, and DOCA-salt groups were significantly less than the PRA values for the sham-water group. Plasma concentrations of Na^+ were slightly less ($P < 0.05$) in the sham-salt group than in the other three groups of rabbits. Plasma K^+ concentrations were significantly ($P < 0.05$) lower in the DOCA-water and DOCA-salt groups than in the other two groups of rabbits. Figure 1 gives the pressor responses to the various doses of NE (logarithmic scale) for the four groups of rabbits in this experiment. The lines in this figure represent the least-squares fit of the data for each group of rabbits to the equation $y = a(\log x)^n$, where y is the change in mean arterial pressure, x is the NE dose, and a and n are constants. The DOCA-salt group of rabbits had significantly ($P < 0.01$) greater increases in mean arterial pressure in response to NE at doses of 100 through 1200 ng/min/kg, than did the other three groups of rabbits; the remaining groups had very similar pressor responses to NE at all doses.

Experiment 2. The cardiovascular values prior to NE infusion for the rabbits in this experiment are summarized in Table II. The values for total peripheral resistance (TPR) are expressed in the arbitrary units that result from dividing the mean arterial pressure in mm Hg, by the cardiac output in ml/min/kg of body wt. There were no significant differences between the two groups for any of the initial measurements. Table III summarizes the changes in the cardiovascular values during infusion of NE at 800 ng/min/kg for both groups of rabbits. During NE infusion the mean arterial pressure and TPR increased

TABLE I. VALUES FOR INITIAL AND FINAL BODY WEIGHTS, MEAN ARTERIAL PRESSURE, PLASMA RENIN ACTIVITY, AND PLASMA CONCENTRATIONS OF Na^+ AND K^+ IN THE FOUR GROUPS OF RABBITS IN EXPERIMENT 1

Group	Initial body weight (kg)	Final body weight (kg)	Mean arterial pressure (mm Hg)	PRA	Plasma Na^+ (meq/liter)	Plasma K^+ (meq/liter)
Sham water	3.02 ± 0.08	3.11 ± 0.06[a]	91 ± 4	5.5 ± 0.7	143 ± 0.9	4.1 ± 0.1
Sham salt	3.10 ± 0.09	3.14 ± 0.08	87 ± 5	2.9 ± 0.4[b]	138 ± 0.6[b]	4.1 ± 0.2
DOCA water	3.28 ± 0.16	3.11 ± 0.11	93 ± 2	0.8 ± 0.3[b]	145 ± 1.1	2.1 ± 0.2[b]
DOCA salt	3.15 ± 0.10	2.86 ± 0.14[a]	92 ± 7	0.4 ± 0.3[b]	143 ± 1.0	2.0 ± 0.1[b]

Note. Values are means ± SEM for six rabbits per group. Plasma renin activity (PRA) values are ng of generated angiotensin I per hr of incubation, per ml of plasma.

[a] $P < 0.05$ that the final body weight is different from the initial body weight.

[b] $P < 0.05$ that the value is different from the value in the sham-water group.

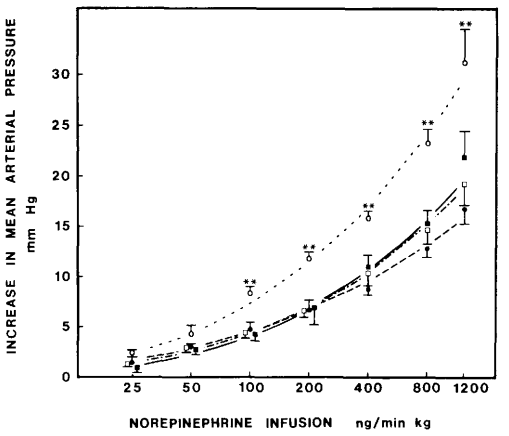

FIG. 1. Increases in mean arterial pressure (mm Hg) in response to infusions of norepinephrine (ng/min/kg body wt, logarithmic scale) in four groups of rabbits in Experiment 1. Black squares and solid line = sham-water group. White squares and dot-dashed line = sham-salt group. Black circles and dashed line = DOCA-water group. White circles and dotted line = DOCA-salt group. Values are means ±SEM for six rabbits per group. Lines represent best-fit of the data for each group to the equation $y = a(\log x)^n$. $^{**}P < 0.01$ that the value is significantly greater than the corresponding value for the other three groups.

in both groups of rabbits; however, comparing the magnitudes of these changes, the DOCA-salt group showed significantly ($P < 0.05$) greater increases than did the sham-water group. Cardiac output was unaltered by the NE infusion.

Experiment 3. Mean arterial pressure in this DOCA-salt group of rabbits averaged 95 ± 3 (SEM) mm Hg, which was not different from the values observed for the DOCA-salt rabbits in Experiment 1. After 30 min of infusion of the AII antagonist the mean arterial pressure averaged 97 ± 4 mm Hg. Mean arterial pressure increased by an average of 31 mm Hg in response to the injection of 300 ng of AII prior to infusion of the AII antagonist, but after 30 min of infusion of [Sar^1, Ile^8] AII the pressor responses to this dose of AII were completely abolished. Likewise, no pressor responses to this dose of AII were observed at the conclusion of the experiment. The pressor responses to the various doses of NE for these DOCA-salt rabbits receiving [Sar^1, Ile^8] AII are illustrated in Fig. 2. For comparison, the dose–response curve for the DOCA-salt rabbits from Experiment 1 (i.e., not receiving the AII antagonist) also is plotted in this figure. As can be seen, these two dose–response curves are very similar, and there are no significant differences between these two groups in the pressor responses to any of the doses of NE. The pressor response to NE at 800 ng/min/kg prior to infusion of the AII analog averaged 22 ± 1 mm Hg; during infusion of [Sar^1, Ile^8] AII the pressor response to this dose of NE again averaged 22 ± 2 mm Hg.

Experiment 4. The values for mean arterial pressure were very similar between the donor and recipient rabbits in each phase of the experiment. As shown in Fig. 3, prior to cross circulation the pressor responses to NE in the DOCA-salt donor rabbits averaged 24 ± 2 mm Hg, which was significantly ($P < 0.01$) greater than the pressor response of 10 ± 1 mm Hg in the normal recipient rabbits. Following the blood cross circulation, the pressor responses to this dose of NE in the recipient rabbits were significantly greater at 5, 15, and 30 min than was the pressor response to this NE dose prior to cross circulation in this group, but by 60 min following cross circulation the pressor response was not different from the pressor response prior to cross circulation. When sham-operated rabbits were used as donors and normal rabbits were used as recipients, the pressor responses were essentially the same in the two groups prior to cross circulation, and the

TABLE II. CARDIOVASCULAR VALUES PRIOR TO NOREPINEPHRINE INFUSION IN SHAM-WATER AND IN DOCA-SALT RABBITS IN EXPERIMENT 2

Group	Mean arterial pressure (mm Hg)	Cardiac output (ml/min kg)	TPR
Sham water	90 ± 4	191 ± 15	0.49 ± 0.04
DOCA salt	91 ± 3	187 ± 14	0.49 ± 0.02

Note. Values are means ± SEM for six rabbits per group. Values for TPR were obtained by dividing mean arterial pressure (mm Hg) by cardiac output (ml/min kg) for each rabbit. There were no significant differences between the two groups for any values.

TABLE III. CHANGES IN CARDIOVASCULAR VALUES DURING NOREPINEPHRINE INFUSION IN SHAM-WATER AND IN DOCA-SALT RABBITS

Group	Δ Mean arterial pressure (mm Hg)	Δ Cardiac output (ml/min kg)	Δ TPR
Sham water	+12 ± 1[a]	+4 ± 4	+0.05 ± 0.02[a]
DOCA salt	+20 ± 1[a,b]	−15 ± 8	+0.17 ± 0.03[a,b]

Note. Values are means ± SEM for six rabbits per group. Norepinephrine dose was 800 ng/min/kg of body wt.

[a] $P < 0.05$ that the change was significantly different from 0.

[b] $P < 0.05$ that the degree of change in the DOCA-salt group was greater than in the sham-water group.

pressor responses to NE in the recipients were not altered by the cross circulation of blood.

Discussion. Previous studies have shown that rats (7) and pigs (8) treated with DOCA developed increased vascular responses to pressor substances. The present study demonstrated that rabbits treated with DOCA and given a salt solution to drink for 14 days developed increased pressor responses to NE, whereas rabbits given only DOCA or only the salt solution failed to develop pressor hyperresponsiveness to NE. These exaggerated pressor responses in the DOCA-salt rabbits were not due to increases in cardiac output but were accompanied by pronounced increases in TPR, presumably due to increased contraction of arteriolar smooth muscle cells; thus, these DOCA-salt rabbits exhibited vascular hyperresponsiveness as well as pressor hyperresponsiveness. The DOCA-salt rabbits, as well as the DOCA-water and the sham-salt rabbits, had mean arterial pressure values that were similar to those for the sham-water control group. The changes in mean arterial pressure in response to NE in these four groups of rabbits, therefore, represented changes from approximately the same basal level.

Expansion of body fluid compartments, especially the plasma and extracellular fluid volumes, may occur in DOCA-salt animals, and the pressor and vascular hyperresponsiveness in these animals may be secondary to this volume expansion. Recent studies by Tajima *et al.* (9) found that DOCA-salt rats have expansions in plasma volume and interstitial fluid volume when compared to controls, whereas rats treated with DOCA alone or given a high sodium intake alone did not have increases in these body fluid compartments. In the present study, the finding of pressor hyperresponsiveness to NE in DOCA-salt rabbits and the failure to find pressor hyperresponsiveness in rabbits treated with DOCA alone or with salt alone suggests that body fluid volume expansion may be necessary for the production of pressor hyperresponsiveness following DOCA-salt treatment. In keeping with this contention are the recent experiments from this laboratory (10) in which rabbits infused with isotonic saline at 1.5 to 1.8 ml per minute for 24 hr had exaggerated pressor and vascular responses to NE. Pamnani *et al.* (11) reported that tail arteries from acutely saline-loaded rats had decreased ouabain-sensitive rubidium uptake, indicating a decreased activity of the Na^+–K^+ pump; similarly, decreased ouabain-sensitive rubidium uptake was seen in tail arteries from DOCA-salt rats (12). Because NE uptake by the sympathetic nerve terminals is sodium and potassium dependent and is inhibited by ouabain (13), Haddy and co-

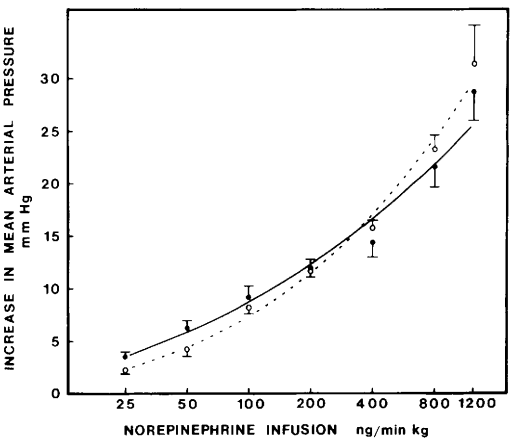

FIG. 2. Increases in mean arterial pressure (mm Hg) in response to infusions of norepinephrine (ng/min/kg body wt, logarithmic scale). White circles and dotted line = DOCA-salt rabbits. Black circles and solid line = DOCA-salt rabbits receiving [Sar^1, Ile^8] angiotensin II. Values are means ±SEM for six rabbits per group. Lines represent best-fit of the data for each group to the equation $y = a(\log x)^n$.

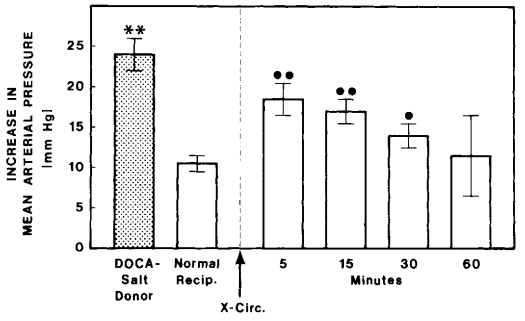

FIG. 3. Increases in mean arterial pressure (mm Hg) in response to iv infusions of norepinephrine at 800 ng/min/kg body wt in DOCA-salt donor rabbits (shaded bar) and in normal recipient rabbits (clear bars) before cross circulation (X-Circ.) and in normal recipient rabbits at 5, 15, 30, and 60 min after cross circulation of blood with donors. Values are means ±SEM for six rabbits in each group. $^{**}P < 0.01$ that the pressor responses were greater in the donors than in the recipients prior to cross circulation. ●● = $P < 0.01$ and ● = $P < 0.05$ that the pressor responses in the recipients were greater after cross circulation than before cross circulation.

workers (14) have suggested that in volume-expanded forms of hypertension there may be decreased NE uptake, producing an accumulation of NE in the synaptic cleft, resulting in hyperresponsiveness to pressor agents. In keeping with this hypothesis was the earlier observation of decreased uptake of labeled NE in tissues of DOCA-salt hypertensive rats (15).

Angiotensin II has been shown to play a role in mediating pressor hyperresponsiveness in some renal hypertensive and prehypertensive animal models in which PRA and plasma AII levels are not elevated. Previous studies from this laboratory have found that 3-day two-kidney, one-clip rabbits (2) and 3-day one-kidney, one-clip rabbits (1) had increased pressor responses to NE; in each of these models, in which arterial pressure and PRA were normal, the pressor hyperresponsiveness was abolished by [Sar^1, Ile^8] AII. A recent study which provided further support of a role for AII in pressor hyperresponsiveness in renal prehypertensive rabbits found that in 3-day one-kidney, one-clip rabbits the enhanced pressor responses to NE were alleviated by the angiotensin converting enzyme inhibitor, captopril (16). The pressor hyperresponsiveness was restored in these captopril-treated rabbits by the iv infusion of AII at doses sufficiently low that they resulted in below-normal plasma concentrations of AII. It has been observed (1) that one-kidney, one-clip rabbits with renal artery stenosis of over 30-days duration and hypertension had accentuated pressor responses to NE, and this pressor hyperresponsiveness was attenuated by [Sar^1, Ile^8] AII, despite PRA values being below normal.

The finding of low values for PRA in DOCA-salt animals does not rule out the possible participation of AII in the pressor hyperresponsiveness in this model. However, the finding from the present study that [Sar^1, Ile^8] AII does not diminish the exaggerated pressor responses to NE in DOCA-salt rabbits provides evidence that AII probably does not have a function in mediating pressor hyperresponsiveness in this model. Therefore, the mechanisms involved in pressor hyperresponsiveness in DOCA-salt rabbits are probably different from those involved in pressor hyperresponsiveness in the renal models.

The studies of Hinke (17) provided an indication that a circulating humoral factor may be involved in mediating pressor hyperresponsiveness in DOCA-salt animals. In perfused ventral caudal arteries from tails from normal rats, it was seen that the addition of serum from DOCA-salt hypertensive rats to the perfusion solution increased the responsiveness of the vessels to pressor substances. The present study provided strong evidence in support of the contention that the pressor hyperresponsiveness in DOCA-salt animals is mediated by a hormonal factor. The cross circulation of blood between DOCA-salt donor rabbits and normal recipient rabbits resulted in the transfer of pressor hyperresponsiveness to the normal recipients. The pressor hyperresponsiveness in these normal recipient rabbits did not result simply from perturbations in the biological system due to the cross-circulation procedures, as the cross circulation of blood in this manner between sham-operated donor rabbits and normal recipient rabbits did not alter the responses in the recipients. The DOCA-salt rabbits undoubtedly had high plasma levels of DOCA which was transferred to the recip-

ient rabbits during blood cross circulation. However, DOCA has a rather slow onset time, as there is a delay of at least 30 min before vascular injections of DOCA affect urinary electrolyte excretions, and the maximal effects do not occur for at least an hour (18), whereas in the present study, after blood cross circulation with DOCA-salt donors, the recipient rabbits exhibited maximal pressor hyperresponsiveness by 5 min, and the effect subsided by 60 min. Thus, the humoral factor mediating pressor hyperresponsiveness following cross circulation with DOCA-salt rabbits in this study probably was not DOCA, but was some fast-acting humoral substance.

Cross-circulation experiments of a similar design between saline-loaded donor rabbits and normal recipient rabbits have shown that the pressor hyperresponsiveness in saline-loaded donors can be transferred to the normal recipients (10), indicating that a humoral factor also is involved in mediating the pressor hyperresponsiveness in this model. Haddy (19, 20) has suggested that natriuretic hormone may be the circulating factor producing the alterations in rubidium uptake and in Na^+-K^+-ATPase activity in conditions of expansion of body fluid volumes, because natriuretic hormone is present in increased amounts in plasma of volume-expanded animals (21) and because natriuretic hormone suppresses sodium pump activity (22). That natriuretic hormone may produce pressor hyperresponsiveness was suggested by the studies of Plunkett *et al.* (23), in which it was observed that a partially purified preparation from plasma of saline-loaded dogs had both natriuretic and vasoconstrictor potentiating activity.

Recent studies have suggested that a digitalis-like substance may be involved in mediating pressor hyperresponsiveness in volume-expanded situations. It has been shown by Gruber *et al.* (24) that plasma from saline-loaded dogs contains a substance that cross-reacts with antibodies to digoxin. Also, treatment of human subjects with digoxin was seen to increase the pressor responsiveness to NE and to AII (25), and ouabain was reported to potentiate NE-induced contractions of the *in vitro* rabbit aorta (26). The experiments of Kojima *et al.* (27) suggested that a digoxin-like substance may be involved in DOCA-salt hypertension; these workers observed a reduction in arterial pressure when anti-digoxin antibodies were administered to DOCA-salt hypertensive rats.

It is of interest that an earlier study from this laboratory (28) found that blood cross circulation with 3-day one-kidney, one-clip donor rabbits and normal recipients resulted in the transfer of pressor hyperresponsiveness to the normal recipients. In these experiments, however, the administration of [Sar^1, Ile^8] AII to the recipient rabbits immediately following the cross circulation prevented the pressor hyperresponsiveness from occurring in the recipients. Because this AII antagonist did not diminish pressor hyperresponsiveness in DOCA-salt rabbits or in saline-loaded rabbits, it is likely that a different hormonal factor is involved in mediating pressor hyperresponsiveness in DOCA-salt and in saline-loaded rabbits than the humoral factor mediating pressor hyperresponsiveness in renal artery stenosis rabbits.

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