Hemodynamic Responses to DOCA in Young Pigs

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SUMMARY Hemodynamic variables were measured in 20 young pigs; thirteen received subcutaneous implantations of desoxycorticosterone acetate (DOCA) impregnated in Silastic strips, seven received implants of Silastic strips alone and served as controls. No salt was added to the standard diet of either group. Mean arterial pressure (MAP) rose in a regular pattern in the DOCA-treated pigs, reaching on the average a level significantly greater than that of the control group 48 hours after the implantation. Pressure continued to rise, reaching a plateau 38% above that of the preimplant value 2 weeks later. In some pigs the MAP elevation was caused by an increase in cardiac output (CO); in others it was caused by an increase in total peripheral resistance (TPR). An increase in central venous pressure occurred in many DOCA-treated pigs regardless of whether the increase in MAP was caused by an increase in CO or in TPR. The results indicate that it is arterial pressure per se that is the regulated variable in this model of mineralocorticoid hypertension. The regulating system, whether it resides in the kidney or in the central nervous system, elevates pressure by effecting increases in either CO or TPR. (Hypertension 1: 591-597, 1979)

KEY WORDS • desoxycorticosterone (DOCA) • hypertension • cardiac output • total peripheral resistance • mean arterial pressure • central venous pressure

In the otherwise unsettled field of hypertension research, it is generally agreed that in the chronic form of the disease the sustained elevation in mean arterial pressure (MAP) results from an increase in total peripheral resistance (TPR). Reports differ, however, as to whether the initiating cause of the elevated pressure is an increase in TPR or an increase in cardiac output (CO). Some evidence has suggested that the process may start as an increase in CO, and then, through an autoregulatory mechanism, shift to an increase in TPR. Recent investigators have failed to corroborate this sequence, and find that the increased MAP from the outset may be caused by an increased TPR.

In the current study, CO and TPR were determined during the development of desoxycorticosterone acetate (DOCA) hypertension in the young pig.

Evidence concerning the hemodynamic event responsible for the onset of hypertension was sought. The initiating determinant of the increase in MAP was found in some animals to be an increase in TPR; in others it was an increase in CO. Preliminary observations of some of these findings have been reported earlier.

Methods

Care and Instrumentation

These studies were initiated on 30 young male feeder pigs (Yorkshire and Chester White), but for reasons given below data are presented on only 20. The pigs were kept in 4' × 4' metabolic cages and given tap water and food ad libitum. Their average daily food intake was 1.3 kg Purina Pig Growena, which contains about 140 mEq of sodium per kg.

Following 1–10 days in the laboratory, each pig was subjected to a thoracotomy (pentobarbital anesthesia) in which an electromagnetic flow probe (Zepeda Instrument Company, Seattle, WA) was placed around the ascending aorta, and a Tygon catheter (id:od = 1.02 mm:1.78 mm) was inserted into the arch.
of the aorta just distal to the flow probe, using the Herd-Barger technique. Approximately 1 week later a laparotomy was performed in which the right kidney was removed; a similar Tygon catheter was inserted into the inferior vena cava and advanced toward the heart, where its tip was left in the central venous pool.

Catheters and leads from the electromagnetic flow probe were brought out through the skin and tied to a Tygon-sheathed copper wire which was looped under the skin on the left side at the level of the eighth rib. A reinforced canvas jacket protected the exteriorized catheters and leads.

**Daily Measurements**

Arterial and venous pressures and CO were monitored daily. The pig was strapped on his right side to a plywood board, weighed, and moved to the monitoring equipment. Although the pig often squealed and kicked when he was placed on the board and strapped down, he rapidly became quiet and on occasions went to sleep while the measurements were being made. The zero reference level for measuring arterial and venous pressures was halfway between the board and the top of the pig’s left shoulder. These two pressures, as well as pulsatile and mean CO, were recorded on a Grass Polygraph. For CO, the zero flow baseline was taken to be the flat, diastolic portion of the pulsatile aortic flow curve. The quotient of MAP divided by CO was used as TPR.

At termination of chronic study, a block of tissue including the aorta and flow probe was excised. The probe was calibrated so that the recorded pen deflections from daily measurements could be converted to actual flow units. Flow calibrations were usually linear, and averaged about 200 ml·min⁻¹·mm⁻¹ pen deflection. The flow probes from three animals could not be calibrated; in one case the probe eroded through the aorta terminally, and, in the other two, technical postmortem problems precluded calibration.

Cardiac output data from these three pigs could not be given in absolute terms (table 1), but were included as percent changes in daily recorded pen deflection (table 1 and figure 1).

**DOCA Administration**

Approximately 2 weeks after the laparotomy, when the pigs were in good health (see below) and when their hemodynamic variables had stabilized, 20 experimental pigs received subcutaneous implantations of DOCA (Sigma Chemical Co., 100 mg/kg) impregnated in Silastic (Dow Corning Corp.) strips, one part DOCA to two parts Silastic by weight; 10 control pigs received implantations of Silastic strips without DOCA. The implantations were made in the left flank with the animal under light thiamylal sodium (Surital) anesthesia. The pig was usually back and eating in his pen 1 hour after the induction of anesthesia.

**Analysis**

Hemodynamic data were analyzed and presented in three ways: 1) as group means on the 16th and 33rd days after implantation (table 1); 2) as group means on each of the first 17 days after implantation (figs. 1 and 2); and 3) as individual values for six pigs over the first 16 days (fig. 3).

Data were discarded if a catheter or flow probe was inoperative (two pigs, 28 remaining), or if the pig was sick, as indicated by body temperature and food intake. If a pig’s food intake decreased by over 50% for 2 or more days in succession, hemodynamic data from that pig for those days were discarded. For this reason data from 11 pigs were unacceptable (17 remaining) during the first 16 days. By the 33rd day, data from seven previously healthy pigs were similarly not acceptable, whereas three pigs, not acceptable at 16 days, had recovered and were included in the group means for the 33rd day. In all, data from 20 pigs were used. Preimplantation values for the group of 13 pigs analyzed at 33 days were nearly identical to those for the 17 pigs presented at 16 days, and are therefore not displayed in table 1.

Pressure data were used only if recordings were normally pulsatile; CO recordings were used only if the pulsatile tracing had a stable diastolic baseline that could be set to null pen deflection. Average values presented in figure 1 are for only those days in which all the 11 hypertensive or six control pigs yielded reliable data; a similar criterion was met for data presented in figure 2.

Because there were differences between pigs in the magnitude of the hemodynamic variables at the time of implantation, the hemodynamic responses of each pig to the implantation were expressed as the percent change from a pre-implantation value for that pig. This could not be done for central venous pressure (CVP) because pre-implantation values ranged from 0 to 9 mm Hg, and a given percent change from these different baselines would not have equivalent physiological significance; therefore, absolute values for CVP and for changes in CVP are presented in table 1 and figures 2 and 3. Pre-implantation values for MAP, CVP, and heart rate (HR) were the averages of values over a stable pre-implant period (usually 6 days). Since the pigs were growing rapidly, CO increased during the pre-implantation period. In order to determine a pre-implantation value for this variable, a linear regression of time versus the stable values of CO before implant was both calculated and independently estimated by two investigators. The intercept of this line with the day of implantation was used to determine an appropriate pre-implantation value. Calculated and estimated values usually agreed within 2%. The pre-implantation value used for TPR was the quotient of the pre-implantation value for MAP divided by that for CO.

All values are reported as mean ± sem. One-tailed, unpaired Student’s t tests were used to compare all data between the experimental and control groups on the 16th and 33rd days after implantation. These days
Results

Hemodynamic data obtained from 13 DOCA-treated and seven control pigs are summarized in Table 1. Changes are shown between pre-implantation values and those obtained 16 and 33 days after implant. It is evident from the weight changes that the pigs were growing rapidly, and that increase in weight continued at about the same rate during these two post-implant periods. There was no significant difference in weight gain between the control and the DOCA-treated groups. Mean arterial pressures of the two groups were similar before implantation. In the control group, this value was not different on either of the subsequent observation days. In the DOCA-treated pigs MAP rose uniformly so that by the 16th day it was 38.0 ± 2.3% above the pre-implant value. During the next 17 days there was only a minimal further increase in pressure reaching 40.1 ± 4.0% above the pre-implant level by the 33rd day. Thus the first of these post-implant periods is one of a rapid increase in pressure; the second is one of a maintained plateau of pressure.

Absolute data for cardiac output are presented for only the 17 pigs for which the flow probe could be calibrated. Uncalibrated data from the remaining three pigs are included in the relative data as percent changes in Table 1 (also in Figures 1 and 3; see below). In the control group CO increased with age; the increase during the second observation period was not as great as that during the first. Cardiac output in the DOCA-treated animals increased more rapidly than did that in the controls and this greater increase seemed to continue through the second observation period.

| Table 1: Hemodynamics, Age, and Weight of Control and DOCA-Hypertensive Pigs* |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                            | Pre-implant                  | Post-implant                |                              |
|                            | Absolute                    | 16 days                     | % Change from pre-implant    | % Change from pre-implant    |
|                            | Control (16)                 | Control (11)                | DOCA (11)                    | Control (11)                |
|                            | Weight (kg)                 | 33.2 ± 1.4                  | 37.0 ± 2.5                  | 99.7 ± 1.9                  | 116.0 ± 5.0                 |
|                            | Age (days)                  | 106.0 ± 5.0                 | 43.1 ± 1.8                  | 99.7 ± 1.9                  | 116.0 ± 5.0                 |
|                            | MAP (mm Hg)                 | 100.5 ± 3.4                 | 136.9 ± 2.6               | 132.5 ± 7.1                | 130.8 ± 2.9                |
|                            | CO (l·min⁻¹)                | 5.54 ± 0.32                 | 6.80 ± 0.49               | 5.64 ± 0.48               | 5.5 ± 0.3                  |
|                            | TPR (PRU)                   | 18.4 ± 1.0                  | 22.1 ± 1.5               | 20.6 ± 1.5              | 18.6 ± 1.5                 |
|                            | HR (min⁻¹)                  | 123.7 ± 4.0                 | 150.0 ± 3.6              | 123.5 ± 7.1              | 124.0 ± 3.6                |
|                            | CVP (mm Hg)                 | 5.3 ± 1.2                   | 5.5 ± 1.3               | 5.3 ± 1.2              | 5.3 ± 1.2                 |
| *Results are expressed as group means ± SEM. Pre-implantation values are chosen and measurements are taken as in the text (see Methods). DOCA = deoxycorticosterone acetate; MAP = mean arterial pressure; CO = cardiac output; TPR = total peripheral resistance; HR = heart rate; CVP = central venous pressure; PRU = peripheral resistance units, mm Hg · l⁻¹ · min⁻¹. |
| † = p < 0.05.                 |                            |                            |                            |                            |
| ‡ = p < 0.001.               |                            |                            |                            |                            |
| §Absolute changes are in mm Hg. |                            |                            |                            |                            |

were used because they typify the ends of the initiating phase of hypertension and of an equivalent period of stable hypertension. Differences between values for control and experimental groups are significant at the p < 0.05 level only if specifically stated.
FIGURE 1. Average arterial hemodynamic variables of control and DOCA hypertensive pigs from 5 days before until 17 days after implanting either DOCA in Silastic or Silastic alone (control). Mean arterial pressure (MAP) was measured via aortic indwelling catheters; cardiac output (CO), by electromagnetic flowmeter; and total peripheral resistance (TPR) was calculated as the ratio of MAP to CO. Average percent changes ± SEM of DOCA-treated and control pigs are shown for those days when data from all animals in each group are complete. MAP increased regularly, whereas rises in both CO and TPR contributed variably to the initiation and maintenance of the hypertension. Since CO increases and TPR decreases with growth in the control animals, the effect of DOCA on these variables is best perceived by noting the difference between the control and DOCA-treated groups.

Calculated TPR in the control group decreased as the animals gained weight and the CO rose. In the DOCA-treated pigs TPR increased 15.2 ± 6.4% by the 16th post-implant day in spite of the increase in weight and CO; however, it declined as the pigs gained weight during the following plateau period at an elevated pressure.

There was no difference between HR of animals in the control group and those in the DOCA-treated group on the 16th or 33rd post-implant days. As the MAP of the treated group was rising, at no time was the average HR of this group less than that of the control.

Central venous pressure did not change in the control group in either of the two observation periods. In the DOCA-treated animals it was significantly elevated on the 16th day post-implant, but not at the 33rd day.

Changes in MAP and its determinants on a day-to-day basis during the first observation period are shown in figure 1. A clear difference between DOCA-treated and control animals was seen only in the case of MAP. The DOCA-treated animals had an average percent change in MAP that was significantly higher than that of controls 48 hours after implantation. Although there appeared to be a tendency for both CO and TPR to be greater in the DOCA-treated animal than in the control, these differences were not as consistently significant as that of the MAP's. Central venous pressure (fig. 2) did not change with time in the control group, but increased during the first week following DOCA implantation.

In figure 3, three representative pigs, A, B, and C, were selected for presentation because their increases in MAP were caused primarily by an increase in CO. An additional three (D, E and F) were selected in which pressure elevations were caused by an increase in TPR. It is evident in figure 3 that although there was a uniform pattern of MAP elevation in both the "cardiac output pigs" and the "total peripheral resistance pigs," the hemodynamic cause of the pressure elevation was clearly different. Of the remaining seven DOCA-treated pigs, the pressure elevations in four were caused by increases in CO, in one by a rise in TPR, and in two by a mixture of increases in both CO and TPR. There appeared to be no difference in the elevations of CVP in the "cardiac output pigs" and in the "total peripheral resistance pigs."

Discussion

Excess mineralocorticoid is known to produce a sodium-dependent hypertension both clinically and in the experimental animal. The mechanism responsible for the elevated arterial pressure is not understood. In the current study the hemodynamic properties of a new experimental model of mineralocorticoid hypertension in pigs are characterized. This model has the advantages inherent in large animals, and provides information arising from comparative pathophysiological observations on a species not previously studied. For example, it is the first model to develop hypertension so rapidly in response to DOCA administration without supplemental sodium. Although young rats on standard diets become hypertensive following large doses of DOCA, they do so much more slowly. Based on the observation that young rats develop DOCA-induced hypertension much more readily than do old rats it is probable that the youth of our pigs contributed to the consistency with which DOCA produced hypertension in them.
Our pigs developed a very regular pattern of elevated arterial pressure following the subcutaneous implantation of DOCA. In most pigs the pressure had commenced to rise 48 hours following this implantation. Mean arterial pressure rose steadily so that by the end of the second week it was consistently over 30% higher than that of the control pigs. The consistency of this pressure elevation has been of value in the consideration of possible hemodynamic mechanisms responsible for it.

No such consistency was observed in changes in the two hemodynamic determinants of the increase in MAP, CO and TPR (fig. 1). In two of the animals the implantation of DOCA was followed by an increase in...
both CO and TPR; in seven the elevated MAP was caused primarily by an increase in CO, whereas in four it was caused mainly by an increase in TPR (fig. 3). Two recent hemodynamic studies on mineralocorticoid hypertension in dogs have emphasized similar variability in the hemodynamic factor responsible for the increase in MAP. In both of these studies it was found that when myocardial function was depressed by beta adrenergic blockade, the increase in MAP was unaffected, and that the increase in pressure then resulted entirely from an increase in TPR. Two older studies, often referred to in support of the predominant role of CO in initiating hypertension, actually give evidence supporting a variability in CO rise after both volume loading, and in early perinephric hypertension. Variability in the hemodynamic abnormality (either increase CO or TPR) responsible for hypertension has also been observed in clinical states of mineralocorticoid excess.

These observations argue that neither CO nor TPR is the primary variable affected by the perturbation induced by the administration of DOCA; in contrast the regularity of the pattern with which arterial pressure is elevated makes it appear that MAP per se is the variable that is regulated.

These hemodynamic observations fail to support the possibility that this model of hypertension results from an initial increase in CO that gives way to an autoregulatory response that increases TPR ("Guyton's hypothesis"). In the pig the initial elevation in MAP is caused by an elevation in either CO or TPR, or both. There was no consistent pattern in which one of these variables increases before the other. It is evident that in those pigs in which the elevation in MAP was caused by an increase in CO, this increase persisted throughout the observation period. There was no tendency for the tissues to regulate their perfusion by increasing TPR and then for TPR to become the cause of the elevated MAP. Furthermore, it is not likely, in those pigs in which the elevation of MAP was caused by an increase in TPR, that CO rose first and then decreased before our first daily measurements could detect it. Cardiac output should not drop until MAP is high enough to increase cardiac afterload or to cause an elevated renal fluid excretion. Mean arterial pressure was not significantly raised until 2 days after implant. In six animals hemodynamic measurements were made at 8-hour intervals following DOCA implantations and no initial increase in CO was observed in pigs in which the pressure rise was caused by an increase in TPR.

Recent studies on the DOCA-hypertensive pig have demonstrated an increase in vascular reactivity to norepinephrine and to angiotensin II as early as 2 days following the implantation of DOCA. This is probably due to an increase in vascular smooth muscle sensitivity. These observations are compatible with the possibility that the increase in mean arterial pressure may be initiated by an increase in vascular smooth muscle sensitivity, which causes a greater constriction in response to normally occurring stimuli, thereby increasing TPR.

The evidence presented in the preceding paragraphs indicates that there is an elevated CVP which should cause an increase in CO, and that there is also an increase in TPR. Perhaps the same type of vascular changes occurs in both veins and arteries; when the venous effect predominates, CO increases and when that in resistance vessels predominates, TPR is increased. This unifying hypothesis is supported by observations that similar changes occur in arteries and veins in hypertension. Pamnani and Overbeck have observed increased water, sodium, and potassium in both venous and arterial walls. This common effect suggests that excess mineralocorticoids might act directly on either venous or arterial smooth muscle.

However, this hypothesis is not in accord with the following observations: 1) recent studies have failed to show any direct enhancement of vascular smooth muscle's contractile activity by the action of desoxycorticosterone; (2) interventions in the rat's central nervous system with either 6-hydroxydopamine or localized lesions prevent an increase in mean arterial pressure in response to DOCA and salt administration; and, 3) following the administration of 6-hydroxydopamine to the rat's cerebral ventricle, mineralocorticoid and salt failed to increase vascular reactivity. These observations indicate that the central nervous system may mediate the hypertensive effect of DOCA.

The consistency of the elevation in arterial pressure in the absence of consistent changes in CO or in TPR in the pig model of mineralocorticoid hypertension argues that it is pressure per se and not CO or TPR that is regulated. Whether the primary regulating system is the kidney or the central nervous system, it has access to an increase in either cardiac output or total peripheral resistance in order to effect the elevation in MAP.

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References

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