PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 163, 540–546 (1980)

Reversal of One-Kidney, One-Clip Hypertension by Unclipping: The Renal, Sodium-Volume Relationship Reexamined (40811)

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In 1949, Byrom and Dodson (1) demonstrated that removal of the clip (unclipping) constricting the renal artery of the one-kidney, one-clip hypertensive rat of 4.5 to 32 months' duration was attended by the arterial pressure receding to normal values within 24 hr. The Byrom experiment has been reproduced for various purposes by several workers (2–8). We wish to record our results with this procedure, utilizing three approaches, namely, (i) allowing urine to flow freely to the outside of the body; (ii) preventing the outward flow of urine by ureterocaval anastomosis (UCA); and (iii) preventing the outward flow of urine by ureteral ligation (UL). All three approaches were tested with and without the addition of a saline load given intravenously. Our approaches in the rat differ from those previously recorded in three major respects: (i) the use of the saline load, (ii) the continuous recording of the mean arterial pressure (MAP) after the manipulations of the renal artery clip, and (iii) the use of the three procedures (free urine flow, UCA, and UL) in the same experimental design. The results, we believe, support the concept of a nonexcretory antihypertensive function of the kidney that, in part, modulates the effect of the extracellular fluid volume on the arterial pressure.

Materials and methods. Male Wistar rats, 9 to 10 weeks old, weighing 200 to 250 g, were subjected to right nephrectomy and the application of a silver clip to the left renal artery under pentobarbital anesthesia (30 mg/kg, ip) as previously described (9). The clip is stable, rectangular, and made to have a fixed gap by doubling on itself. The clip gap was 0.25 mm. Four to twelve months later, when the hypertensive state had been well established at 184 ± 1.6 mm Hg (all designations consist of mean ± SEM), the animals were used for the various approaches. At that time, the body weight was 415 ± 20 g.

Preliminary arterial pressure was obtained by the tail-cuff method, previously described (10). One week prior to use, indwelling aortic and vena caval catheters emerging at the back of the neck were implanted under pentobarbital anesthesia. This method has also been previously described (10). Thereafter, the mean aortic pressure (MAP) was determined by connecting the aortic catheter to a Statham P23Dc transducer and a Grass Model 7 polygraph for 30 min. The caval catheter was used for the saline infusion when this was interposed.

Unclipping procedure. The procedure was conducted under light pentobarbital anesthesia. The anesthetic was injected (30 mg/kg, ip) while the MAP was monitored continuously. A transient depression of the MAP often occurred as the anesthetic action reached a maximum (average 25 mm Hg), lasting an average of 30 min. The abdominal incision was made and the clip was exposed. As soon as the MAP recovered completely from its depressed state, i.e., returned to the preanesthetic level, and while the anesthesia was light, the clip was either removed (test group) or manipulated as a sham procedure (control group).

The clip was removed as follows. The rectangular clip (9) was exposed, and the end opposite the site of the lodgement of the renal artery was severed, using a pair of sharp scissors. The upper plate became free and fell off. The remaining U-shaped section was sprung apart and carefully teased away from the renal artery. This is a tedious process as tissue is present about the artery. Rarely, however, was the artery torn. Following unclipping or the sham maneu-

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ver, the abdominal incision was closed quickly with continuous 4-0 silk at the linea alba and with multiple skin clips at the skin margin. The animal was then placed upright in a retaining wire cage. The MAP was monitored continuously for the necessary time interval (to the time of normalization of the MAP, or a comparable period).

The UCA was accomplished by means of a U-shaped polyethylene tube (PE 10, 5 cm long, with a 45 degree angle tapering at each end). The ureter was double ligated near the urinary bladder and severed between the ligatures. The ureter was punctured with a 27-gauge needle near the ligated distal end. The flared end of the tube was introduced into the lumen of the ureter for ~3 cm and fixed in place by means of two 5-0 silk ties. The vena cava was cleared free of connective tissue. The straight end of the tube was introduced into the vena cava above its origin as one would to perform a venipuncture. It was extended into the vein for 2.5 cm. No sutures were used at this end of the anastomosis. The bottom of the U-shaped portion of the tube was tucked into the subperitoneal tissue for fixation, making certain both sides of the tube were parallel to the vein. In each UCA case, at the end of the experiment, the caval catheter was removed under light anesthesia and by direct vision to determine that urine flow was free, i.e., to determine that no obstruction existed. The latter was considered very important as obstruction would be tantamount to ureteral ligation.

After unclipping, the animals were placed in a restraining wire cage in which they remained quiet for the duration of the experiment. Groups 1 and 2 received no food or water; Groups 3, 4, and 5 received the saline load, as indicated below, but no food or water by mouth.

There were five groups.

Group 1. Unclipping plus free urine flow to the outside (n = 18): There were nine test and nine control animals. During the 3 hr of this experiment, the total urine flow and Na excretion were measured. The Na and K concentration were measured by flame photometry (Instrument Laboratories, Watertown, Mass.).

Group 2. UCA plus unclipping (n = 19): There were 9 test and 10 control animals.

Group 3. Saline infusion plus unclipping and free flow of urine (n = 9): A saline load of 20 ml was injected subcutaneously. The anesthetic was injected, and just before removal of the clip, an additional 5 ml of saline was injected intravenously. The clip was removed and a saline infusion (154 meq Na/L) was begun at the rate of 5.1 ml/hr for 20 hr, using a Sage pump (Model 351). The urine was allowed to flow freely and was collected through a clean glass funnel into a clean bottle. The input and output of Na, K, and water were determined. Body weight was determined before and at the end of the experiment.

Three animals, receiving the same saline load, were sham operated and the MAP and water-electrolyte balance were monitored for the same period of time (20 hr).

Group 4. UCA plus an intravenous saline load amounting to 1.25–2.5% of the body weight for one subgroup and 5% of the body weight for another subgroup (n = 21): The ureter was anastomosed to the vena cava, and this was followed by an intravenous saline load amounting to either 1.25–2.5% of the body weight (4–9 ml) (n = 5) or 5% of the body weight (18–20 ml) (n = 8). Then

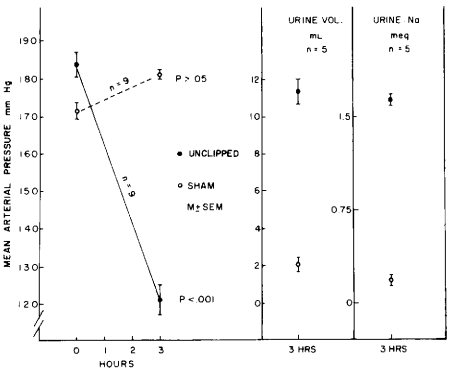

FIG. 1. The results of unclipping, plus a free urine flow, are shown (Group 1). The solid circles represent results following unclipping, and the open circles the results following the sham operation. The P values compare data before and after the renal manipulations. As the MAP receded following unclipping, a brisk diuresis-natriuresis occurred.

the clip was removed and the MAP was monitored. Sham operated controls had the UCA and received the 5% body weight saline load intravenously (n = 8).

Group 5. UL plus an intravenous saline load amounting to 2.5% of the body weight (8–9 ml) (n = 15): The ureter was dissected free near the bladder, double ligated, and severed between ligations. The proximal end was tucked into the nearby tissues. Then the saline load was given intravenously and either the clip was removed or sham manipulated. In addition, on two occasions, the ureter was ligated, the saline load was not injected, and the clip was removed.

Results. Group 1 (Fig. 1). In all cases (n = 9), the MAP became normal within 3 hr. The control group (n = 9) had no change in MAP. At the same time as the MAP was normalized, there was a prominent diuresisnatriuresis (urine flow 6 × controls; Na excretion, about 8 × controls).

Group 2 (Fig. 2). The MAP receded to normal within an average of 20 hr (n = 9). Sham-operated controls (n = 10) had no change in MAP.

Group 3 (Fig. 3). The MAP receded to normal in 15–20 hr (n = 9), similar to results following the UCA maneuver. Five animals had a minor weight gain, four had a minor weight loss. Statistically, there was no change in weight for the group ($P > 9$).[1]

There was a positive Na and water balance (input 18 ± 0.6 and output 12.8 ± 0.7 meq of Na; input 117 ± 3.7 and output 87.2 ± 4.8 ml of water). Since there was no K intake and some K was excreted in the urine, the K balance was slightly negative (input 0 and output 3 ± 0.1 meq of K).

The three animals subjected to the sham maneuver had no change in MAP (202 ± 1.4 mm Hg before, 205 ± 2.4 mm Hg after). At the same time, Na and water balances were positive (Na: in 15.7 ± 2.9, out 9.5 ± 3.2; water: in 102 ± 19, out 64 ± 23 ml). The weight did not change significantly (395 ± 3.3 vs 413 ± 48). The K balance was negative (−3.8 ± 1.4 meq).

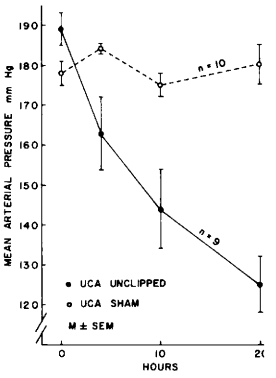

FIG. 2. The results of unclipping plus UCA are shown (Group 2). Unclipping plus UCA necessitated 20 hr for the MAP to reach normal.

Group 4 (Fig. 4). As can be seen in the figure, both unclipped subgroups had their MAP recede to normal levels but this required 45–50 hr. Sham-operated controls had no change in their MAP for 45 hr. Between 45 and 50 hr, five of the eight sham-operated animals died and displayed pulmonary edema at autopsy.

Group 5 (Fig. 5). Both the test and control groups displayed a slight depression of the MAP over 45 hr (about 20 mm Hg, remaining considerably above normal) but there was no difference between the two groups ($P > 0.9$). On the two occasions in which the ureter was ligated, no saline was given, and the clip was removed, there was no change in MAP over 45 hr (the MAP remained near 200 mm Hg).

Discussion. Unclipping the one-kidney, one-clip hypertensive rat of 4–12 months' duration plus free urine flow (Group 1) was attended by diuresis-natriuresis and a rapid decline of the MAP. These findings confirm those of Ledingham and Cohen (2) and Liard and Peters (4). In the absence of the loss of Na and water to the outside of the body, i.e., the UCA maneuver (Group 2), the recession of the MAP to normal required 20 hr. This is comparable to the results of Floyer (3) in which, by isolated measurements, the arterial pressure reached normal within 24 hr. Thus,

[1] It is emphasized that rats from this colony without food or water for the same period of time lose an average of 29 g, presumably from passage of feces and insensible water loss.

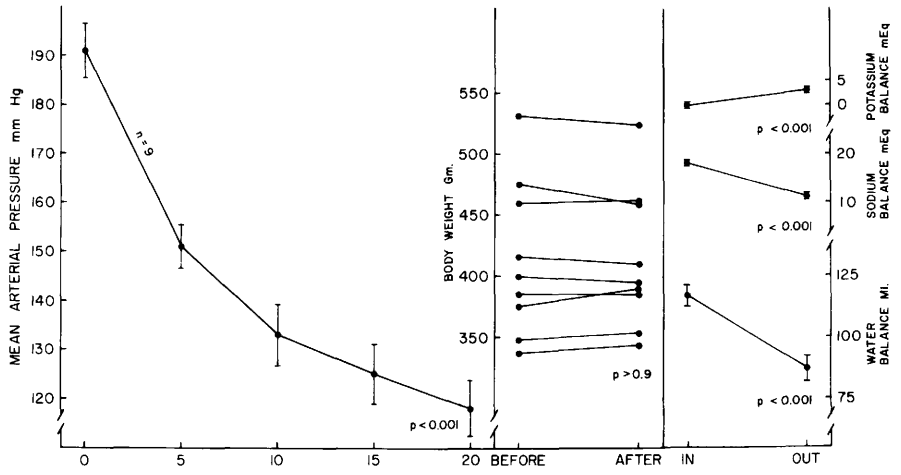

FIG. 3. In these experiments, a saline load was introduced (25 ml), then the clip was removed and an intravenous infusion was instituted (5.1 ml/hr) for the duration of the experiment (20 hrs) while urine flowed freely. As indicated by the far left panel, the MAP receded from ~190 to ~120 mm Hg in 20 hr. The middle panel indicates minor weight changes, while the far right panel shows a positive water and Na balance ("In" indicates input by the infusion, and "Out" indicates urinary output). There was a minimal negative K balance. The changes in MAP were similar to those following UCA.

diuresis-natriuresis appeared to accelerate the antihypertensive action following unclipping but was not essential for it to occur. Conversely, it may be taken that a normal ECFV status slowed down the antihypertensive effect of unclipping the one-kidney, one clip hypertensive rat.

Unclipping and a free flow of urine to the outside of the body, plus a saline infusion that maintained a positive Na-volume balance (Group 3), was also attended by a recession of the MAP to normal within 15–20 hr. Under these conditions, there were minimal changes in body weight. The net

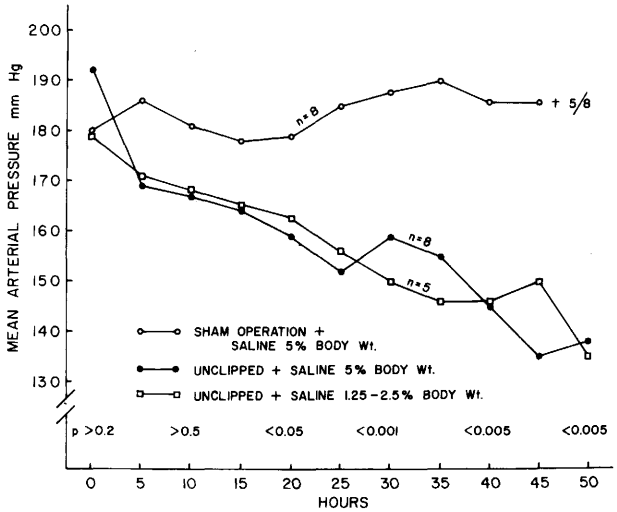

FIG. 4. Following the UCA, a saline load was introduced intravenously (equivalent to 1.25–2.5% of the body weight in one subgroup and 5% of the body weight in another subgroup) (Group 4). The MAP receded to the normal range by 45–50 hr. The sham-operated controls had no change in MAP over 45 hr; thereafter, most of these animals died (five out of eight, as shown by the cross).

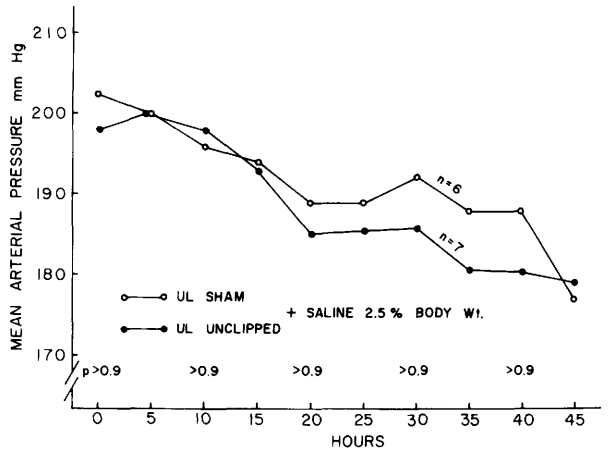

FIG. 5. Ureteral ligation (UL) plus a saline load intravenously (equivalent to 2.5% of the body weight), plus unclipping, gave the same results as the sham-operated group (Group 5). Both groups displayed a slight recession of the MAP over 45 hr (from ~200 to ~180 mm Hg) but normalization did not occur.

effect for the group was no significant change in body weight. The results of this experiment are in keeping with those of Ledingham and Cohen (2) and Neubig and Hoobler (8), in which the urine formed following unclipping was collected and returned to the body by the intravenous route, thereby preventing the loss of body fluids; despite this maneuver, the arterial pressure receded to normal.

The similar time course for lowering the MAP of Groups 2 and 3 support the interpretation that the lowering of the MAP associated with the UCA was not due to the introduction of antihypertensive substances from the kidney through the ureterovenous anastomosis since, in Group 3, urine flowed to the outside of the body.

When a Na-volume load was added to the UCA, followed by unclipping, the time required for normalization of the MAP was extended to 45–50 hr. Thus, three time intervals were observed for the MAP to reach normal following unclipping, depending on the state of the body fluids; namely, 3 hr when body fluids were contracted (Group 1), 15–20 hr when body fluids were maintained near normal (Groups 2 and 3), and 45–50 hr when body fluids were expanded (Group 4).

The implications of these experiments, as previously considered by different groups of workers (2–8), are that the unclipping procedure allowed the kidney to exert an antihypertensive action. This action is not confined to the loss of Na and volume to the outside of the body, *it is not confined to contraction of body fluids*. The kidney, then, appears to exert a nonexcretory antihypertensive function (11). The latter hypothesis entertains the possibility that the kidney secretes an antihypertensive hormone.

It appears that in order for the kidney to exert this antihypertensive function, the state of the renal hemodynamics must be intact. This interpretation is indicated by the results following UL plus a saline load (Group 5). UL plus a saline load was not followed by normalization of the MAP within 45 hr. Prior experience indicated that UL, plus a saline load, compromised the state of the renal papilla, leading eventually to papillary necrosis (12, 13). Whether the necrosis results from pressure on the vasa recta due to the hydronephrosis, as we postulated previously (12), or whether the intrarenal generation of vasoconstrictor agents, such as angiotensin (14) or thromboxane (15), is involved in the papillary damage remains moot.

The renal papilla and its renomedullary interstitial cells (RIC) are capable of exerting an endocrine-type antihypertensive ac-

tion (16–20). In view of this established action, it could be that maintaining the functional potential of the RIC by maintaining the hemodynamic integrity of the kidney in Groups 1–4 allowed the antihypertensive action mediated by these cells to assert itself. Conversely, a compromise of the action of the RIC, associated with UL and the Na-volume load, might explain the failure of the MAP, under these circumstances, to normalize.

Factors, other than a renomedullary effect, may be involved when hydronephrosis is sustained. These include a reduction in renal blood flow and glomerular filtration pressure and a decrease in glomerular filtration (21, 22). An elevation of vasoconstrictor substances may occur, not only within the kidney, as just mentioned (14, 15), but angiotensin may be elevated systemically (23). Thus, the state of the cardiac output and vascular reactivity may effect the arterial pressure, under these circumstances.

The net effect of these results is to support the view that the nonexcretory and possibly hormonal antihypertensive renal function, in part, modulates the effect of the extracellular fluid volume on the arterial pressure. The specifics of how this modulation transpires remain unknown.

Lucas and Floyer (24, 25) generated data in support of a factor from the kidney that presumably controls interstitial gel compliance. According to this hypothesis, the absence of this factor, as in the renoprival state, decreases compliance of the gel, thereby increasing the intravascular volume when a volume load is added, and causing an elevated arterial pressure through hemodynamic effects (26, 27). The presence of this factor, as in the UCA preparation, increases the interstitial gel compliance, according to these workers, and protects the vascular system from an expanded volume and an elevated arterial pressure. This attractive concept is in need of verification. One attempt to do so (28) led to the conclusion that the renal effect was on the heart rather than on plasma volume expansion or decreased venous compliance.

Transplants of a pure tissue culture of RIC lower the MAP to normal or near normal by two actions, i.e., an early acute depressor effect (in some, but not in all experiments) and a slower effect transpiring often over 24 hr (29), but on some occasions requiring a few days' time for the maximum effect. This antihypertensive action of RIC mimics that of the UCA preparation plus unclipping. In terms of the experiments herein reported, it is noteworthy that the antihypertensive effect of RIC occurs in Na-volume loaded hypertensive states (29, 30). Nonprostanoic lipid extracts of fresh renal medulla and also of cultured RIC (29) exert an antihypertensive action similar to that of RIC and the UCA-unclipping maneuver. It is not known, however, whether the latter lipids are mediators of the antihypertensive action of the RIC. At present, these lipids remain the main candidates for the hormone-like action of the RIC.

Summary. The kidney exerts an antihypertensive function that appears, in part, to countervail the prohypertensive actions of Na-volume loads. This function seems to require a normal, or near normal, renal hemodynamic state. The time course for this renal action is dependent on the state of the body fluids, rapid with contracted body fluids, intermediate with normal body fluids, and prolonged with body fluid expansion.

The similarities in the antihypertensive action of UCA and transplants of RIC suggest a commonality of action between these procedures. It is suggested that the common denominator between these procedures is the antihypertensive action of the RIC. The mediator(s) of this action is unknown. The most prominent candidate(s) for the mediation are lipids extracted from renal medulla and cultured RIC.

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Received September 25, 1979. P.S.E.B.M. 1980, Vol. 163.