Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 114 OCTOBER 1963 No. 1

SECTION MEETINGS

IOWA
State University of Iowa

October 30, 1962
November 27, 1962
January 29, 1963
February 26, 1963
March 26, 1963
April 30, 1963
May 28, 1963

## Renal Hemodynamic Effects of Hypertonic Mannitol Infusions.* (28570)

WILLIAM E. BRAUN AND LAWRENCE S. LILIENFIELD
*Cardiovascular Research Laboratory, Department of Medicine, and Department of Physiology and Biophysics, Georgetown University Medical Center, Washington, D.C.*

Hypertonic mannitol solutions have been reported to be of value in a wide variety of clinical settings. Their use in the prevention of oliguria associated with cross-clamping of the aorta(1) prompted us to study further the renal hemodynamics during hypertonic mannitol infusions in normotensive and hemorrhagic hypotensive dogs.

*Methods.* A total of 15 dogs was used. In all dogs, following induction of anesthesia and laparotomy, renal clearances of inulin and paraaminohippuric acid and extraction ratios of paraaminohippuric acid were determined. In 6 dogs these determinations were again made during mannitol infusion and again during hemorrhagic hypotension with the mannitol infusion maintained. Two of these dogs were also studied following retransfusion. Two additional dogs were studied during mannitol infusion alone. In another group of 5 dogs, studies were made during hemorrhagic hypotension and again during subsequent mannitol infusion. Two additional dogs were studied during hemorrhagic hypotension and subsequent retransfusion.

Male and female mongrel dogs weighing 10 to 22 kg were anesthetized with sodium pentobarbital (25 mg per kg). Arterial blood pressures were continuously monitored from a femoral artery by means of a strain gauge transducer. After laparotomy the left renal vein was exposed so that the ovarian or spermatic vein draining into it could be ligated and divided. The left renal vein was then catheterized via a femoral vein. The left ureter was also catheterized. In some dogs

* Supported in part by grants from U.S.P.H.S.

the right renal vein and ureter were used. With a constant infusion pump, paraaminohippuric acid (PAH) and inulin were administered into a femoral vein, after priming doses had been given. Levels of 1 to 3 mg% of PAH and 20 to 40 mg% of inulin were achieved. A 5% dextrose in water solution was infused into a jugular vein at a rate of 3 to 4 cc per minute until the mannitol infusion was begun. Mannitol was infused as a 20% solution at 5 to 6 ml per minute after a priming dose of 3 g. Three or more consecutive PAH clearances ($C_{PAH}$) and inulin clearances ($C_{IN}$) were measured simultaneously, and PAH extraction determinations were made on blood drawn midway through

TABLE I. Renal Effects of Mannitol Infusion Before and During Hemorrhage.

| | Dog | Column 1<br>Control | Column 2<br>Mannitol | Column 3<br>Mannitol during hemorrhage | Column 4<br>Transfusion and mannitol |
|---|---|---|---|---|---|
| $E_{PAH}$ | 1 | .76 | .59 | .56 | .30 |
| $C_{PAH}$ | | 59 | 64 | 43 | 51 |
| $C_{IN}$ | | 18 | 13 | 9 | 13 |
| RPF | | 78 | 104 | 74 | 150 |
| RBF | | 142 | 168 | 117 | 200 |
| $E_{PAH}$ | 2 | .62 | .31 | .46 | .25 |
| $C_{PAH}$ | | 34 | 33 | 28 | 28 |
| $C_{IN}$ | | 12 | 9 | 6 | 7 |
| RPF | | 55 | 104 | 55 | 103 |
| RBF | | 95 | 160 | 79 | 147 |
| $E_{PAH}$ | 3 | .73 | .40 | .34 | |
| $C_{PAH}$ | | 36 | 25 | 16 | |
| $C_{IN}$ | | 17 | 5 | 6 | |
| RPF | | 49 | 59 | 44 | |
| RBF | | 100 | 89 | 59 | |
| $E_{PAH}$ | 4 | .74 | .47 | .53 | |
| $C_{PAH}$ | | 41 | 45 | 32 | |
| $C_{IN}$ | | 18 | 16 | 9 | |
| RPF | | 55 | 93 | 60 | |
| RBF | | 100 | 138 | 80 | |
| $E_{PAH}$ | 5 | .57 | .30 | .26 | |
| $C_{PAH}$ | | 40 | 27 | 20 | |
| $C_{IN}$ | | 13 | 8 | 8 | |
| RPF | | 70 | 90 | 66 | |
| RBF | | 113 | 125 | 85 | |
| $E_{PAH}$ | 6 | .51 | .21 | .11 | |
| $C_{PAH}$ | | 31 | 24 | 14 | |
| $C_{IN}$ | | 10 | 4 | 4 | |
| RPF | | 61 | 97 | 105 | |
| RBF | | 127 | 159 | 142 | |
| $E_{PAH}$ | 7 | .97 | .80 | | |
| $C_{PAH}$ | | 44 | 47 | | |
| $C_{IN}$ | | 12 | 8 | | |
| RPF | | 45 | 57 | | |
| RBF | | 100 | 95 | | |
| $E_{PAH}$ | 8 | .89 | .76 | | |
| $C_{PAH}$ | | 87 | 85 | | |
| $C_{IN}$ | | 24 | 13 | | |
| RPF | | 98 | 112 | | |
| RBF | | 178 | 170 | | |
| $E_{PAH}$ | Mean & S.E. | .72 (± .05) | .48 (± .08) | .38 (± .07) | .27 |
| $C_{PAH}$ | | 46 (± 6.6 ) | 44 (± 7.7 ) | 25 (± 4.5 ) | 39 |
| $C_{IN}$ | | 15 (± 1.6 ) | 9 (± 1.5 ) | 7 (± .8 ) | 10 |
| RPF | | 64 (± 6.2 ) | 90 (± 7.3 ) | 67 (± 8.6 ) | 126 |
| RBF | | 119 (±10.2 ) | 138 (±11.4 ) | 94 (±12.3 ) | 173 |

$C_{PAH}$, $C_{IN}$, RPF, and RBF are expressed in ml/min/one kidney.

each clearance period. Clearance periods ranged from 5 to 20 minutes. Renal plasma flow (RPF) was calculated from the formula:

$$RPF = \frac{V(Cu - Cv)}{C_A - C_V}$$

, where V is urine flow, and Cu, Cv, and $C_A$ are PAH concentrations in urine, renal vein, and renal artery plasma, respectively. The extraction of PAH was corrected for the amount of PAH that enters red blood cell water in dogs(2). Inulin determinations were performed according to the method of Schreiner(3); PAH, according to the method of Smith(4).

Mean arterial pressure in all dogs during the control period was in the range of 100 to 160 mm Hg. When hemorrhagic hypotension was induced, mean arterial pressure was lowered to 75 mm Hg by removing between 18 and 36 ml of blood per kg. In some experiments it was necessary, during mannitol administration, to continue removing blood in order to maintain a relatively fixed level of hypotension.

When transfusion studies were done, the blood previously removed was retransfused.

*Results.* During the administration of hypertonic mannitol solution to the 8 dogs under anesthesia and during laparotomy (Table I, column 2), there was a consistent fall in $E_{PAH}$ from an average of .72 ± .05 (S.E.) during control periods, to .48 ± .08 (S.E.) during the infusion of mannitol. The $C_{PAH}$ was unchanged on the average, though in 3 dogs there was a slight fall associated with a declining arterial pressure. Renal plasma flow (RPF) rose significantly to 139% of the control value, and RBF, calculated from the RPF and arterial hematocrit, also rose to 116% of control values.

In the 7 dogs in which the effects of hemorrhage alone were investigated (Table II, column 2), there was no significant change in $E_{PAH}$ although $C_{PAH}$ fell markedly to 6%, RPF to 6%, RBF to 5%, and $C_{IN}$ to 11% of control values.

In contrast, in the 6 dogs in which mannitol infusions were started before and continued during the bleeding and hypotensive periods (Table I, column 3), $E_{PAH}$ fell significantly from .65 ± .04 (S.E.) to .38 ± .07 (S.E.) while $C_{PAH}$ fell only to an average of 62% of control values. RPF in these dogs rose to 111% and RBF fell only to 84% of control values. $C_{IN}$, on the other hand, fell to 47% of the control clearance value. There was no significant difference in the total amount of blood withdrawn to maintain the same level of hypotension in the groups without or with the mannitol infusion.

In the 5 dogs in which mannitol was infused after induction of hypotension (Table II, column 3), $E_{PAH}$ was not significantly changed by the hemorrhage or by the mannitol infusion although in one dog (10) $E_{PAH}$ fell markedly after hemorrhage and again further after mannitol. $C_{PAH}$ which had fallen to 8% during hemorrhage rose to 46% of control values. RPF rose during the infusion from 7% to 37% and $C_{IN}$ from 9% during hemorrhagic hypotension to 36% of control values during the mannitol infusions.

In the 2 dogs which were studied during hypotension and then again after retransfusion (Table II, column 4), $E_{PAH}$ remained unchanged and $C_{PAH}$ which had fallen to 4% of control value after hemorrhage rose to 67% after transfusion. RPF rose from 4% to 61% of control and RBF from 4 to 66% of control values. In addition, $C_{IN}$, which had fallen to 16% of control values during hemorrhagic hypotension rose after retransfusion to 73% of the control values.

In the 2 dogs (Table I, column 4), which were retransfused after concomitant hemorrhagic hypotension and mannitol infusions, $E_{PAH}$, which had fallen with the infusion, fell even further after transfusion to 39% of control values while $C_{PAH}$ rose to 35%, calculated RPF to 189% and RBF to 148% of control values. $C_{IN}$ also rose, though only to 67% of the control values.

During mannitol infusion the arterial hematocrit decreased in all dogs. This decrease was very marked in those dogs which had been bled before the mannitol administration (Table III).

*Discussion.* The effects of the hypertonic mannitol infusion on renal hemodynamics under the conditions of these studies were quite marked. In the anesthetized dog during laparotomy alone, the infusions invariably

resulted in a significant fall in renal extraction of PAH associated with a fall in glomerular filtration rate while $C_{PAH}$ was unchanged. Several interpretations of these findings should be considered. The infusions might have had an effect on the proximal tubular epithelial cells so that their ability to transport PAH was altered(5). This could account for the reduced PAH extraction ratio. However, unless cortical plasma flow was simultaneously increased, PAH clearance would have been expected to fall. The fact that PAH clearance did not change significantly while extraction fell could be explained by a rise in cortical plasma flow. A rise in plasma flow with the observed fall in GFR suggests efferent arteriolar dilatation. A second explanation is that there was an increased intratubular pressure associated with mannitol diuresis which decreased effective filtration pressure and thereby decreased GFR. Either a rise in RBF or a fall in hematocrit, as in the hemorrhaged dogs, could account for the fall in $E_{PAH}$(6,7). A third interpretation also does not require postulating an effect of the mannitol infusion on transport of PAH. If

TABLE II. Renal Effects of Mannitol Infusion After Hemorrhage.

| | Dog | Column 1<br>Control | Column 2<br>Hemorrhage alone | Column 3<br>Mannitol after hemorrhage | Column 4<br>Transfusion alone |
|---|---|---|---|---|---|
| $E_{PAH}$ | 9 | .91 | .81 | .86 | |
| $C_{PAH}$ | | 46 | 11 | 34 | |
| $C_{IN}$ | | 18 | 5 | 11 | |
| RPF | | 51 | 14 | 39 | |
| RBF | | 84 | 22 | 52 | |
| $E_{PAH}$ | 10 | .99 | .57 | .22 | |
| $C_{PAH}$ | | 45 | 4 | 7 | |
| $C_{IN}$ | | 22 | 1 | 2 | |
| RPF | | 45 | 7 | 31 | |
| RBF | | 110 | 12 | 42 | |
| $E_{PAH}$ | 11 | .63 | .83 | | .77 |
| $C_{PAH}$ | | 104 | 1.5 | | 56 |
| $C_{IN}$ | | 30 | 6 | | 21 |
| RPF | | 165 | 2 | | 73 |
| RBF | | 281 | 4 | | 143 |
| $E_{PAH}$ | 12 | .81 | .81 | | .68 |
| $C_{PAH}$ | | 34 | 5 | | 37 |
| $C_{IN}$ | | 14 | 1 | | 11 |
| RPF | | 42 | 6 | | 54 |
| RBF | | 81 | 10 | | 95 |
| $E_{PAH}$ | 13 | .73 | .74 | .62 | |
| $C_{PAH}$ | | 86 | 1 | 15 | |
| $C_{IN}$ | | 30 | .2 | 3 | |
| RPF | | 118 | 1 | 24 | |
| RBF | | 197 | 1 | 29 | |
| $E_{PAH}$ | 14 | .68 | .79 | .75 | |
| $C_{PAH}$ | | 100 | 3 | 57 | |
| $C_{IN}$ | | 21 | 1 | 9 | |
| RPF | | 147 | 4 | 76 | |
| RBF | | 277 | 7 | 101 | |
| $E_{PAH}$ | 15 | .91 | .90 | .87 | |
| $C_{PAH}$ | | 37 | 4 | 32 | |
| $C_{IN}$ | | 20 | 2 | 14 | |
| RPF | | 41 | 4 | 37 | |
| RBF | | 76 | 6 | 52 | |
| $E_{PAH}$ | Mean & S.E. | .81 (± .05) | .78 (± .04) | .66 (± .12) | .72 |
| $C_{PAH}$ | | 64 (±11.2 ) | 4 (±1.3 ) | 29 (± 8.6 ) | 46 |
| $C_{IN}$ | | 22 (± 2.2 ) | 2.5 (± .8 ) | 8 (± 2.3 ) | 16 |
| RPF | | 87 (±20.6 ) | 5 (±1.7 ) | 41 (± 9.0 ) | 63 |
| RBF | | 159 (±35.7 ) | 8 (±2.6 ) | 55 (±12.2 ) | 119 |

$C_{PAH}$, $C_{IN}$, RPF, and RBF are expressed in ml/min/one kidney.

TABLE III. Changes in Arterial Hematocrit and Urine Flow in Dogs Subjected to Hemorrhage with Mannitol Infusion.

| Dog No. | Hematocrit | Urine flow (ml/min/one kidney) |
|---|---|---|
| | Control | |
| 1 | 45 | .6 |
| 2 | 42 | .1 |
| 3 | 51 | .1 |
| 4 | 45 | .1 |
| 5 | 38 | .3 |
| 6 | 52 | .5 |
| | Mannitol and hemorrhagic hypotension* | |
| 1 | 37 | 3.5 |
| 2 | 29 | 2.0 |
| 3 | 26 | 2.0 |
| 4 | 25 | 2.4 |
| 5 | 23 | 4.0 |
| 6 | 26 | 3.2 |

* Mean arterial blood pressure 75 mm Hg.

it is assumed that PAH is completely extracted by the proximal tubule cells in a single passage through the peritubular capillaries, then the observed fall in $E_{PAH}$ with an unchanged $C_{PAH}$ when the mannitol solution was administered would mean that there was an increased blood plasma perfusion of "non-functioning" tissue. The magnitude of the fall in extraction suggests either arterial-venous shunting or a relatively increased perfusion of the renal medulla. It should be noted that the renal medulla is supplied with blood via the juxtamedullary glomeruli and that the efferent arterioles of these glomeruli have a larger diameter than those associated with cortical nephrons(8). Dilation of efferent arterioles throughout the cortex and medulla might result in a proportionately greater increase in medullary blood flow with a consequent fall in the extraction ratio of PAH.

The effects of hemorrhagic hypotension in this study on $C_{PAH}$ and $E_{PAH}$ were no different than have been reported from many other laboratories(9,10,11). At a mean arterial blood pressure of 75 mm Hg, sufficient urine flow was present through the ureteral catheter to provide urine for collection. The marked reduction in $C_{PAH}$ without significant changes in $E_{PAH}$ and the somewhat lesser reduction in $C_{IN}$ imply renal vasoconstriction, especially constriction of the efferent arterioles. When hypertonic mannitol was infused during hemorrhagic hypotension, there occurred a substantial increase in RBF and glomerular filtration rate with arterial pressure maintained the same. Although some of the increase in glomerular filtration can be accounted for by the marked plasma protein dilution with decrease in colloid osmotic pressure which must occur when hypertonic mannitol solution is given, the accompanying, and even more marked, increase in RBF can best be accounted for by dilation of constricted arterioles, predominantly efferent arterioles.

The apparent return of $C_{PAH}$ and $C_{IN}$ toward control values following the mannitol infusion (Table II, column 3) could have been an experimental artifact. It is possible that during the marked reduction in urine flow accompanying hemorrhagic hypotention not all of the extracted PAH or filtered inulin was carried into the collecting system. Under these circumstances, determinations of $C_{IN}$ and $C_{PAH}$ would yield artificially low values. During osmotic diuresis with increased urine flow and better collection, $C_{IN}$ and $C_{PAH}$ would appear increased. Although this possibility cannot be definitely ruled out without direct measurement of renal blood flow, the fact that $C_{IN}$ and $C_{PAH}$ did not both increase to the same extent with the infusion tends to negate this hypothesis. In addition, in all clearance measurements made during hypotensive periods and afterward at least 3 clearance periods were utilized with excellent agreement among them. Furthermore, the first urine collections after periods of hypotension were always discarded.

Heinemann, Smythe, and Marks have reported a tendency for return of RBF spontaneously 20 to 50 minutes after hemorrhage with arterial pressures in the range of 90 mm Hg. That this phenomenon cannot account for the effects observed here can be seen from the results observed in the group of dogs in which the mannitol infusion was well established prior to the bleeding. Unlike the dogs receiving only 5% dextrose prior to hemorrhagic hypotension, the dogs receiving hypertonic mannitol failed even to develop marked renal vasoconstriction following hemorrhage (Table I, column 3).

The mechanism whereby the hypertonic

mannitol solution exerts its effect is completely unknown. The possibility that the hypertonic mannitol solution exerts a direct relaxing effect on renal arterioles must be considered. Harvey(12) found that in isolated cold perfused kidneys hypertonic mannitol infusions decreased renal resistance. However at the same time these perfusions always decreased urine flow, an observation quite different from that found in the intact animal kidney and making comparison quite difficult. Recent work of Peters and Brunner (13) confirms the preservation of urine flow with hypertonic mannitol infusions following hemorrhagic hypotension to levels below 50 mm Hg. They further suggest that the site of arteriolar dilatation under those circumstances is preglomerular.

In a preliminary study in this laboratory of non-anesthetized, non-operated human subjects, 20% mannitol infusions given at a rate of 5 ml per minute did not significantly alter renal hemodynamics. The reported clinical usefulness of mannitol solutions in patients undergoing surgery suggests that the infusion may abolish reflex or humoral renal vasoconstriction. In the 2 hemorrhagic hypotensive dogs who were already receiving mannitol, blood transfusion produced a marked increase in renal blood flow, well above control levels. This indicates that the vasodilating effect of the mannitol persisted even when blood pressure was returned to near normal levels, and is not concordant with a hypothesis that mannitol infusions abolish reflex sympathetic renal vasoconstriction. In one dog to which hypertonic mannitol solution was given while epinephrine was being administered intravenously (12.6 μg/min), RPF, which had fallen to 14 ml/min during the epinephrine infusion, rose to 143 ml/min when mannitol was added, without a significant rise in the epinephrine depressed glomerular filtration rate. Additional studies of this kind, as well as investigation of other osmotically active solutions, are now under way, and may shed more light on the underlying mechanisms involved.

*Summary.* The effects of intravenous administration of 5 ml/min of a 20% mannitol solution to operated anesthetized dogs was investigated. In normotensive animals the infusion resulted in a prompt fall in the extraction ratio of PAH, an increase in renal blood flow, and a reduction in glomerular filtration rate. In dogs made hypotensive by bleeding, administration of the mannitol solution, either prevented the marked renal vasoconstriction usually accompanying hemorrhagic hypotension, or restored renal blood flow and to a lesser extent glomerular filtration rate toward control values. The observed effects of the infusion are believed to be the result of renal vasodilation, especially of the efferent arterioles. The mechanism whereby hypertonic mannitol infusion produces this effect is unknown. It is suggested that it operates by blocking humoral vasoconstriction that accompanies anesthesia, trauma, or hemorrhage.

The authors gratefully acknowledge the technical assistance of Mrs. Jean Young, Mrs. Irma Zimmerman and Mr. Thomas Doyle.

1. Barry, K. G., Cohen, A., Knochel, J. P., Whelan, T. J., Jr., Beisel, W. R., Vargas, C. A., LeBlanc, P. C., Jr., *New Eng. J. Med.,* 1961, v264, 967.

2. Smith, H. W., *The Kidney, Structure and Function in Health and Disease,* Oxford Univ. Press, New York, 1951, p162.

3. Schreiner, G. E., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 117.

4. Smith, W. W., Finkelstein, N., Smith, H. W., *J. Biol. Chem.,* 1940, v135, 231.

5. Lehninger, A. L., *J. Biochem.,* 1961, v49, 553.

6. Harth, O., Kreienberg, W., Lutz, J., *Pfluger's Arch. ges. Physiol.,* 1959, v570, 174.

7. Kiil, F., *Nature,* 1961, v189, 927.

8. Smith, H. W., *Principles of Renal Physiology,* Oxford Univ. Press, New York, 1956, p8.

9. Van Slyke, D. D., *Ann. N. Y. Acad. Sci.,* 1947-48, v49, 593.

10. Heinemann, H. O., Smythe, C. D., Marks, P. A., *Am. J. Physiol.,* 1953, v174, 352.

11. Selkurt, E. E., *ibid.,* 1946, v145, 699.

12. Harvey, R. B., *ibid.,* 1960, v199, 31.

13. Peters, B., Brunner, H., *ibid.,* 1963, v204, 555.

Received April 29, 1963. P.S.E.B.M., 1963, v114.