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ADH Effects in Development of Ischemic Acute Renal Failure (40748)

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Results in a dog model which combined hemoglobinuric and renal blood flow reduction processes were interpreted to indicate that antidiuretic hormone (ADH) plays a critical role in the pathogenesis of acute renal failure (ARF) (1). It has also been shown that circulating levels of ADH increase about 40-fold in rats with glycerol-induced ARF (2–4). However, in Brattleboro rats with congenital diabetes insipidus (DI rats) glycerol effectively induced ARF (5). It is not clear, therefore, whether the release of ADH does contribute to the pathogenesis of ARF, or if it is secreted as a consequence of the uremic state. Moreover, in glycerol-induced renal failure, an increase in osmolality secondary to a transfer of glycerol from muscle to blood, in addition to an increase in plasma osmolality produced by muscle destruction, could account for the increase in ADH secretion (6). The increased ADH level could therefore be unrelated to the development of ARF. Another uncontrolled factor would be changes in renal metabolism of ADH during development of ARF.

We have attempted to clarify the possible role of ADH in the pathogenesis of ARF using the ischemic model of ARF in rats. We have shown that: 1. Renal ischemia combined with ADH administration can result in ARF even when the ischemia alone or treatment with ADH alone did not produce ARF; 2. saline administration to maintain high hydration is not effective in preventing hypoxia-induced ARF; 3. renal anoxia of DI rats results in production of ARF.

Methods. Because this work was carried out in two different laboratories, each laboratory used the rat strain with which they had had past experience, and about which they had accumulated information. At the Sheba Medical Center in Israel, Charles River rats (CR) were used exclusively and were obtained from Yokneam, Israel. Wistar rats (W) were obtained from Calgary, California for use in Albuquerque. In addition, DI rats of the Brattleboro strain were grown in the laboratories of the University of New Mexico and kindly supplied by Dr. Helmuth Vorherr and Ute Vorherr. All animals were known to be homozygous for the DI trait from their breeding history. In addition, physiological evidence confirmed the existence of the DI trait. Treatment with ADH in infancy was needed to insure survival, and high fluid intake and urine output was also confirmatory in non-ADH-treated mature animals. Individual strains are indicated as necessary in the results section of this paper.

All animals were allowed food and water *ad libitum* until the time of surgery. At that time the animals weighed between 250 and 350 g. Renal ischemia was induced according to procedures previously described (7, 8). In brief, the animals were anesthetized with ether and the femoral artery was cannulated for administration of saline and drugs. Following laparotomy, the right kidney was removed. The left renal artery was exposed, the kidney was stripped of perirenal fat, and the artery was clamped for 70 min. After clamp removal, the laparotomy was closed. Sham-operated controls were treated in the same way except that the left kidney was manipulated but not clamped. Following recovery from anesthesia, the rats were placed in metabolic cages for 24 hr to allow for the development of azotemia. Urine was collected in a graduate, and volume was measured directly. Urinary creatinine was determined using an autoanalyzer, and emission flame photometry was used for measuring sodium and potassium. At the end of the 24-hr period, the animals were reanesthetized and blood was removed from the inferior vena cava. Blood was analyzed for creatinine using an autoanalyzer. Analysis for urea was done either by autoanalyzer (Israel) or

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by the diacetyl monoxide and thiosemicarbazide method (Sigma Bull. No. 535 in Albuquerque). Although the determining factors are not known, the difference in the strain of rats, differences in geographic sites of the studies, or differences in methods of analysis could result in slight differences in blood urea concentrations. Directional changes were always qualitatively the same. Since strain differences could have been responsible for the quantitative differences, results for all parameters are presented for all strains of animals. Results are expressed as mean ± standard error. Statistical evaluation was carried out using the Student's *t* test. Except where otherwise specifically indicated, all animals received 6 ml of saline per hour for a period of 100 min. Drugs were administered in the infusion solution.

In the first series of studies, the interaction between ADH and ischemia was investigated. Group A was sham-operated controls and group B underwent 70-min ischemia. A third group (Group C) consisted of animals having only 40 min of ischemia. The fourth group (Group D) had the same ischemic time as group C, and the ischemia was followed by 5 U of octapressin administered over a 30-min period. In group E, 11.2 U of ADH alone was administered over 70 min. This procedure simulated a comparable period of reduced blood flow as in renal ischemia animals (Group B).

In the second series of experiments, the effect of an increase in circulatory volume was studied. Rats (Group F) were infused with saline at a dose of 5% of body weight for 40 min before clamping the renal artery in order to increase circulatory volume. The renal artery was clamped for 70 min as in group B. After clamping, the fluid administration was the same as the other groups. In the final series of studies (Group G), ARF was induced in DI rats using 70 min of ischemia. In this group, 12 ml of saline per hour was administered for 100 min.

Results. The interaction between ADH and ischemia. Renal artery clamping for 70 min in uninephrectomized rats (Group B) produces severe acute renal failure in both groups of rats. Serum urea nitrogen increases four- to sixfold from 28.2 ± 3.2 in shams to 122.8 mg% ± 11.2 (CR) and from 21.8 ± 2.3 to 167.3 ± 18.15 (W). Serum creatinine also showed an increase from 1.5 ± 0.1 to 4 mg% ± 0.3 (CR) and from 1.16 ± 0.1 to 4.64 ± 0.37 (W). Thus, 24 hr after declamping the probability that either group of animals has the same serum urea or creatinine concentration is < 0.001. This is an acute nonoliguric renal failure with a 24-hr urinary output as high as the sham-operated controls. The 24-hr urinary creatinine excretion was 2.56 mg ± 0.51 (CR) and 3.48 mg ± 0.87 (WR) which is significantly lower than that of sham-operated rats ($P < 0.001$). The ARF groups had a significantly higher 24-hr urinary sodium ($P < 0.001$), and lower potassium excretion ($P < 0.01$) when compared to the sham controls.

Rats with the renal artery clamped for only 40 min (Group C) did not develop ARF. Twenty-four hours after declamping the serum urea nitrogen, Na and K were not different for the sham controls. The 24-hr urinary excretion of creatinine, Na, K were also similar to the sham-operated rats. Experimental group D, in which 40-min ischemia is followed by infusion of ADH for another 30 min, developed ARF, although less severe than group B with 70-min ischemia. The serum urea nitrogen and creatinine rose to 73.44 mg% ± 11.56 (CR) and 2.09 mg% ± 0.27, respectively, 24 hr after declamping. This was also a nonoliguric ARF. The 24-hr urine volume was 14.2 ml ± 1.67. In this group the urinary 24-hr sodium excretion was significantly higher than in the sham-operated group ($P < 0.01$). The administration of ADH alone for 70 min without previous ischemia of the kidney did not induce ARF (Group E).

The effect of increased circulatory volume (group E). An increase of 5% of body weight with normal saline infusion, during 40 min prior to the 70-min renal ischemia as in group B, did not protect the rats from similar severe ARF as in group B.

Ischemic ARF in DI (Brattleboro) rats (group F). Uninephrectomy and 70-min ischemia of the remaining kidney result in a

TABLE I. EFFECTS OF ADH AND HYDRATION ON PRODUCTION OF ISCHEMIC RENAL FAILURE[a]

Experimental group	SUN mg%		S_{CR} mg%		S_{Na} meq/liter		S_K meq/liter		HCT %	
	CR	W	CR	W	CR	W	CR	W	CR	W
A $n = 6$ (CR) $n = 6$ (W)	28.8 ± 3.2	21.8 ± 2.3	1.5 ±0.1	1.16 ±0.08	138.5 ± 2.2	133.3 ± 2.1	3.9 ±0.2	4.02 ±0.1	38 ± 1.1	39.2 ± 0.75
B $n = 10$ (CR) $n = 11$ (W)	122.8*** ± 11.2	167.3*** ± 18.15	4*** ±0.3	4.64*** ±0.37	139 ± 2.5	131 ± 2	4.7 ±0.2	5.26 ±0.25	35.2 ± 3.2	32.9 ± 1.41
C $n = 7$	28.63 ± 1.75		1.34 ±0.04		139.8 ± 0.77		4.31 ±0.13		38.5 ± 4.1	
D $n = 8$	73.44** ± 11.56		2.09 ±0.27		138.7 ± 0.75		4.11 ±0.13		35.6 ± 2.3	
E $n = 7$		15 ± 0.73		0.97 ±0.11		125.4* ± 2.82		4.19 ±0.12		43.4 ± 0.95
F $n = 9$		153*** ± 18.4		4.32*** ±0.43		128.8* ± 1.1		5.1** ±0.3		33.3 ± 1.8
G $n = 7$ DI rats	111*** ± 8.95		6.31*** ±0.39		134.1 ± 0.67		5.73 ±0.3		43.7 ± 1.5	

Experimental group	V ml/24 hr		$U_{Na} \cdot V$ meq/24 hr		$U_K \cdot V$ meq/24 hr		$U_{CR} \cdot V$ mg/24 hr		BW g	
	CR	W	CR	W	CR	W	CR	W	CR	W
A $n = 6$ (CR) $n = 6$ (W)	10.2 ± 0.7	15.25 ± 4.03	0.29 ±0.05	0.43 ±0.04	1.12 ±0.16	1.32 ±0.14	10.72 ± 1.07	9.72 ±1.57	295 ± 5	261.3 ± 2.2
B $n = 10$ (CR) $n = 11$ (W)	14.3 ± 2.4	15.23 ± 2.63	1.022*** ±0.6	1.39*** ±0.26	0.55** ±0.2	0.46*** ±0.13	2.56*** ± 0.51	3.48*** ±0.85	291 ± 7.2	274.3 ± 5.7
C $n = 7$	9.93 ± 0.67		0.574*** ±0.1		1.89 ±0.185		9.47 ± 0.67		278 ± 6.3	
D $n = 8$	14.2* ± 1.76		0.941*** ±0.11		1.63* ±0.1		6.546* ± 0.48		290 ± 8.1	
E $n = 7$		13.1 ±3.24		0.646 ±0.15		1.381 ±0.19		4.94 ±0.96		284 ± 10.2
F $n = 9$		16.42 ± 6.86		1.79*** ±0.64		0.62** ±0.2		4.42** ±1.1		264.4 ± 1.8
G $n = 7$ DI rats	4.64*** ±2.96		1.5 ±0.74		0.09 ±0.04		1.27*** ±0.94		279.7 ± 18.1	

[a] Group A, sham-operated rat; Group B, 70-min ischemia; Group C, 40-min ischemia; Group D, 40-min ischemia and 30-min ADH, 5-μm total dose; Group E, 70-min ADH, 11.2 μm total dose; Group F, volume expansion 5% saline for 40 min, followed by 70-min ischemia; Group G, DI rats, 70-min ischemia; W, Wistar rats; CR, Charles River rats. The numbers are mean ± SE.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

severe form of ARF in DI rats. The serum creatinine in this group 24 hr after the declamping was the highest of all groups, 6.31 mg% ± 0.39. The rats also had a high hematocrit 43.7 ± 1.5 and a high serum potassium 5.73 meq/liter 0.3. The urine volume in the 24 hr following the induction of ARF was extremely low for this strain of rats, 4.64 ml ± 1.96. They also had a high urinary sodium and an extremely low potassium excretion in the 24 hr following declamping.

Discussion. The observations made in this study were: 1. Subthreshold bouts of ischemia combined with administration of large doses of ADH produces ARF; 2. ADH administration alone does not cause renal failure; 3. ischemic renal failure can be produced in Brattleboro DI rats indicating the ADH is not necessary for induction of ARF; 4. saline expansion at the time of the anoxic insult does not alleviate uremia in this renal model of ARF. As far as we know this is the first demonstration that two different subthreshold stresses can combine to produce ARF.

It is known that production of ARF by renal ischemia requires a minimal duration of reduced blood flow for an adequate time, the severity of the subsequent uremia is dependent upon the duration of the ischemia. In contrast, ADH is neither sufficient or necessary for production of ARF. One cannot produce renal failure with massive doses of ADH, yet ARF can be produced in DI rats. The fact that subthreshold ischemia and ADH in combination can produce ARF indicates that ADH had an effect on the course of ARF even though it is not a critical factor. The interaction could depend on its vasoconstrictor actions. (9) The whiteness of the kidney is most impressive in this respect (unpublished). In group D where the stimuli are administered simultaneously the administration of ADH could serve to prolong effectual ischemic time so that a threshold level for production of ARF was reached. If this explanation is correct, the effects of high ADH may have clinical significance. In some of the common situations leading to ARF in man, such as trauma or surgery accompanied by anaesthesia, high levels of ADH are produced.

If one considers the increase in concentration of serum urea, and creatinine as an index of the severity of ARF, then it would appear that the DI rats had the most intense response. Although urea was not very different from other strains (Table I), S_{Cr} was the highest of any experimental group. Urinary volume was markedly reduced, below that of any group even though polyuria is present in untreated animals. These observations can possibly be interpreted as indicating that the pattern of ARF is different in DI rats. It was noted, but not quantitatively, that these rats did not drink as much as preoperatively (unpublished). It is known that unoperated DI rats have high serum Na and seem to be chronically dehydrated (11). The observation that the urine volume is low in ischemia-induced ARF in this species may also indicate that dehydration per se is a contributing factor to increasing the severity of ARF.

The observation that acute administration of saline at the time of insult had no effect on the future production of ARF is consistent with observations made in other experimental models of ARF (12). The results of these studies are in contrast with those done on humans wherein beneficial treatment for prevention of ARF involves maintenance of body fluid volume (13). One must consider, however, that the latter situation involves treatment of ARF, whereas in the case of fluid administration at the time of insult, prevention of ARF is being attempted. It is probably inappropriate to compare the two situations.

One final point deserves consideration. In groups E and F, S_{Na} was reduced. Such an effect is to be expected in the ADH-treated animals (group E) since this hormone induces retention of water and also can cause enhanced urinary excretion of Na under some conditions. The reason for the reduction in Na in the saline-treated rats with subsequent ischemia does not seem to be readily and rationally explained. Because the difference from controls is small and just reaches significance, this result may be spurious.

Summary. After uninephrectomy and contralateral renal artery clamping for 70 min, severe uremia developed in two different species of rats. Twenty-four hours after

the operation the serum urea nitrogen (SUN) was 122.8 ± 11.2 mg% (CR) and 167.3 ± 18.15 mg% (W). The serum creatinine was 4 ± 0.3 mg% (CR) and 4.64 ± 0.3 mg% (W). Expansion of volume by 5% of body weight just before the ischemic period did not reduce the severity of the renal failure. The administration of antidiuretic hormone for 70 min without renal artery clamping or an anoxic period of only 40 min are not able to produce uremia. A combination of 40 min renal artery clamping followed by 30 min of intravescular administration of ADH resulted in ARF. SUN was 73.44 ± 11.56 mg% 24 hr after surgery and S_{Cr} 2.09 ± 0.27 mg%. Congenital diabetes insipidus rats (Brattleboro) were not protected against ischemic ARF. The SUN was 111 ± 8.95 mg% and serum creatinine was 6.38 ± 0.39 mg% 24 hr after surgery. It is concluded that the absence of ADH does not offer protection against ischemia-induced ARF. However, high ADH levels in combination with a subthreshold renal ischemic period are a contributory factor in the development of this type of experimental ARF.

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