HgCl₂-induced Acute Renal Failure in the Developing Rat

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Summary

The present investigation was undertaken to find the differences, if any, in the pattern of nephrotoxic acute renal failure (HgCl₂, 4.7 mg/kg body weight SC), in the developing rat and its relationship to the renin angiotensin system. No differences in renal cortical renin content were found between 2, 4, and 8 week olds, but plasma renin concentration was highest at 2 weeks and declined with age. Plasma renin was significantly increased in all groups 6 hr after HgCl₂ injection, and the percentage of increase was highest in the 4 week olds. Despite these differences in initial plasma renin and in changes in plasma renin after HgCl₂, the pattern of acute renal failure (as assessed by changes in blood urea nitrogen) was similar in the three groups for the first three days. Subsequently, the 4 and 8 week olds exhibited recovery (blood urea nitrogen began to decline), whereas blood urea nitrogen continued to increase to the fifth day in the 2 week olds. The mortality was highest in this group. No simple correlation was observed between basal renal renin, plasma renin, the increase in plasma renin following HgCl₂ injection, and the pattern or severity of acute renal failure.

Speculation

The delayed recovery of renal function in younger rats may be due to a limitation in their ability to eliminate nephrotoxin imposed by immaturity of both glomerular and tubular function.

The pathogenesis of acute renal failure (ARF) remains controversial despite extensive investigations (2, 12, 21, 33). Almost all the investigations have been performed in mature animals, but inasmuch as the renin angiotensin system has been postulated to play a major role in the pathogenesis of ARF (10-14, 17, 21, 28), the developing animal may be a useful model to study because it is characterized by significant changes in the components of the renin angiotensin system during the course of development in various species, including man (15, 18, 23, 24, 26, 31).

The present investigation was undertaken to find the differences, if any, in the pattern of nephrotoxic ARF (mercuric chloride, 4.7 mg/kg body weight, SC) in the developing rat and its relationship to the renin angiotensin system. A preliminary report of some of our findings has been presented (4).

METHODS

Sprague Dawley rats, bred in our lab so that the exact postnatal ages were known, were studied at 2, 4, and 8 weeks of age according to the protocol shown in Figure 1. Random animals of either sex at various ages were sacrificed by decapitation for baseline measurements of renal cortical renin content (RCRC), plasma renin concentration (PRC), and blood urea nitrogen (BUN).

To follow the course of acute renal failure, the 4- and 8-week-old animals were weaned at 3 weeks of age and placed in metabolic cages for 48 hr before the experiments with continuous access to rodent chow and tap water. Daily weights, fluid intake, and urine

volumes were recorded for the duration of the study. The urine collected for the 24 hr preceding the experiment was analyzed for Na⁺, K⁺, and Cl⁻. On the morning of the experiment, a tail vein blood sample was obtained from the 8-week-old animals for control BUN. Then HgCl₂ was administered SC (4.7 mg HgCl₂ per ml 140 mM NaCl, 1 ml/kg body weight), and BUN determinations were repeated 1, 2, 3, 5, and 7 days later.

Because of the possible deleterious effect of initial blood sampling in the 4-week-old animals, only one-third of this group had a baseline BUN determination. Then the group was divided into 2 subgroups: those that had BUN determinations on days 1, 3, 5, and 7 and those that had BUN determinations on days 2, 5, and 7. Although somewhat higher BUN values were observed in those which had an initial tail vein sample drawn, the results were not significantly different from those which did not.

The 2-week-old pups were allowed to stay with their mothers because the weaning age in rats is approximately 3 weeks. Random animals were sacrificed from the litters for baseline BUN values, and the rest received HgCl₂ as stated above. Because of the difficulty of obtaining serial samples in these small animals, random pups were sacrificed for BUN determinations on days 1, 2, 3, 4, 5, and 7. To follow body weight changes as well as mortality, renal failure was induced in additional litters of 2-week-old pups. Survivors of this group were sacrificed at 7 days for BUN determinations. Mean body weight of this group was compared with that found in other litters of 2-week-old pups which had received NaCl (140 mM; 1 ml/kg) rather than HgCl₂.

Urinary Na⁺ and K⁺ were measured by flame photometry with an internal Li⁺ standard. Cl⁻ was measured by titration with a direct-reading Corning chloride meter. BUN was measured with a General Diagnostic BUN Strate Kit which is based on a modification of the Faucett and Scott method for 0.2-ml samples (35). PRC and RCRC were measured as previously described (6-8).

The *t*-test was used to analyze the significance of any observed differences, and a *P* value exceeding 0.05 was considered not significant when two groups were compared. When more than two groups were compared, only *P* values of less than 0.01 were considered significant. All results are mean ± S.E.

RESULTS

Table 1 shows the basal values of body weight, PRC, RCRC, and BUN in rats that were sacrificed and not given injections of HgCl₂. With respect to PRC, RCRC, and BUN, no sex differences were found in any age group, and the results for these were combined. Basal BUN was highest in the 2 week olds and declined with age. There was no difference between the groups with respect to RCRC, but PRC was significantly higher in 2 week olds than in the other groups, and it too declined with age. However, the difference between 4 and 8 week olds was of borderline statistical significance.

Figure 2 shows the PRC response in the 3 age groups to HgCl₂ injection. In each age group, PRC was significantly elevated 6 hr after the injection. The magnitude of the increase was highest in the 4 week olds: 154% of basal, as compared with 70% (8 week olds) or 47% (2 week olds). In fact, 6 hr after HgCl₂ injection, the

PRC's of the 2 and 4 week olds were not significantly different from each other, but both were significantly higher than that of the 8-week-old rats.

Figure 3 shows the pattern of HgCl_2 -induced ARF, as assessed by mean BUN values on days 1, 2, 3, 5, and 7 after injection. The development of failure during the first 3 days was similar in all 3 groups. Subsequently, 2 patterns emerged. BUN continued to rise in the youngest rats, whereas it declined markedly and significantly in both groups of older rats. Comparing mean BUN's on the fifth day, the 4 and 8 week olds were not significantly different from each other, but both were significantly lower than the 2 week olds. This delayed recovery in the 2 week olds, as assessed by BUN, was also reflected in the number of animals in each group which had BUN values over 100 mg/100 ml on the fifth day: 14 of 27, 7 of 33, and 5 of 31 in the 2, 4, and 8 week olds, respectively. There were declines in BUN in all 3 groups between the fifth and the seventh days, but the marked fall in the youngest group may be somewhat misleading, in that this group had the highest mortality rate, and the BUN values on the seventh day most probably represent the values of the more mildly affected surviving pups.

The mortality at the end of the 7-day period for the 3 groups was 38 of 70 (54.3%), 14 of 46 (30.4%), and 5 of 36 (13.8%) for 2, 4, and 8 week olds.

Figure 4 shows the mean weight changes as percentages of pre- HgCl_2 weights in the 3 groups of rats. As can be seen, the 2 and 4 week olds exhibited a more anabolic pattern than did the 8 week olds.

Table 2 shows the fluid intake, urine volume, and excretion rates of Na^+ , K^+ , and Cl^- , all per 100 g body weight, in the 24 hr preceding HgCl_2 injection. The 4 week olds had higher values for electrolyte excretion than did the 8 week olds, probably reflecting a higher food intake.

DISCUSSION

Most studies of experimentally induced ARF have been carried out in mature animals. Considerable controversy exists about the

role of the renin angiotensin system in the pathogenesis of ARF (2, 10-14, 17, 21, 28, 33). The developing animal should be a useful model to study because there are spontaneous changes in components of the renin angiotensin system during development (15, 18, 23, 24, 26, 31).

Both PRC and plasma renin activity have been reported to be higher in the young and to decline with age in several species including man (15, 18, 23, 24, 26, 31). Our results are in agreement with these studies. The reason for the high PRC in the young is not clear. Both increased rate of release and decreased rate of inactivation have been invoked to explain it (26), but few studies are available in which renin release was measured as a function of age. *In vitro* studies with renal tissue slices of Sprague-Dawley rats suggest that basal renin release is higher in young animals (1). To the extent that changes in PRC reflect changes in renin release, our data indicate that the renin response to a given stimulus (HgCl_2 injection) varies with age. If one examines the degree of change of PRC in response to HgCl_2 , one observes the most marked change in the 4-week-old rats, with the result that, although its basal values were similar to the 8-week-old rats, its post- HgCl_2 values were not significantly different from the 2-week-old rats. Although similar *in vivo* studies at these stages of development in the rat are not available for other stimuli, the release of renin from kidney slices in response to norepinephrine does show a decline with age (1).

Differences in PRC with age do not seem to be due to, or paralleled by, differences in RCRC in our study, as well as in the study of Pohlova and Jelinek (26). However, other investigators have found increases during development (29). These conflicting results may represent either species or methodologic differences.

The significance of these observations in relation to either the severity or the pattern of ARF remains unclear. No definite relationship is seen to exist between the severity of ARF during the first three days after HgCl_2 , as judged by mean BUN values, and the basal PRC, PRC 6 hr after HgCl_2 , or the magnitude of

EXPERIMENTAL PROTOCOL

Fig. 1. ARF was studied in Sprague Dawley rats of 3 age groups: 2, 4, and 8 weeks old. Some rats of each age were sacrificed for measurements of baseline RCRC, PRC, and BUN. The others were given HgCl_2 to induce renal failure. Some of each age group were sacrificed 6 hr later to determine the effect of ARF on PRC, and the others were followed to determine the course of ARF.

PRC (ng AI/ml/hr) control and 6hrs after HgCl_2

Fig. 2. PRC found in rats of different ages (2, 4, and 8 weeks old). PRC was measured in control rats at each age and in other rats 6 hr after induction of ARF with HgCl_2 . Numbers in parentheses, numbers of animals in each group.

Table 1. Basal values at sacrifice of noninjected rats

Age (wk)	Wt (g)	BUN (mg/100 ml)	RCRC ¹	PRC ¹
2 (n = 18)	23.7 ± 0.8 ²	23.8 ± 1.4	9,268 ± 928	52.9 ± 7 (14) ³
4 (n = 21)	66.1 ± 2.9	19.3 ± 1.0	10,101 ± 893	26.9 ± 7 (18) ⁴
8 (n = 17)	208 ± 10.2	18.1 ± 0.7 ³	11,064 ± 1038	20.9 ± 1 (14) ⁴

¹ ng angiotension ± per mg of protein per hr.

² Mean ± S.E.

³ Numbers in parentheses, number of rats.

⁴ Significantly different from values for 2 week olds, $P < 0.01$ maximum.

Pattern of HgCl₂ (4.7mg/kg SQ) induced ARF in the various age groups

Fig. 3. BUN was measured in control rats of ages 2, 4, and 8 weeks and in other rats of the same ages on days 1, 2, 3, 5, and 7 following a SC injection of HgCl₂. See text for statistical significance of any differences between groups. Numbers in parentheses, number of animals in each group.

Fig. 4. Body weights of 2-, 4-, and 8-week-old rats on days 1, 2, 3, 5, and 7 following a SC injection of HgCl₂. Weights are expressed as percentages of pre-HgCl₂ body weights.

Table 2. Intake and urinary output in 24 hr preceding HgCl₂¹

Age (wk)	Wt (g)	Fluid intake (ml)	Urine vol. (ml)	U _{Na} V (mEq)	U _{Cl} V (mEq)	U _K V (mEq)	BUN (mg/100 ml)
4 ² (n = 46)	68.1 ± 1.9 ¹	21.2 ± 1.0	4.7 ± 0.3	0.76 ± 0.05	1.04 ± 0.06	1.3 ± 0.07	19.4 ± 1.3
8 (n = 36)	201.8 ± 6.2	13.2 ± 0.9	6.2 ± 0.6	0.39 ± 0.03	0.48 ± 0.05	0.77 ± 0.05	15.8 ± 0.6

¹ Fluid intake, urine volume, and excretion data are all factored by 100 g body weight per 24 hr.

² All values for 4 week olds are significantly different from those for 8 week olds, *P* < 0.05 at most.

¹ Mean ± S.E.

the increase in PRC. There was indeed a difference between the groups in the pattern of ARF after the third day; the youngest animals exhibited delayed recovery. We do not have PRC measurements during this period of ARF recovery. However, even in the adult rat, the PRC changes at this time are highly inconsistent and difficult to interpret because of the coexistent changes in water and electrolyte balance (12, 19). Moreover, even if high PRC were to be found, it could be a consequence, rather than the cause, of the renal failure. Thus, it remains possible that the renin angiotensin system played some role in the delayed recovery of the 2-week-old group; there is no evidence for or against this possibility. On the other hand, this period in the development is

characterized by spontaneously occurring changes in many parameters of renal function, including maturation of superficial cortical glomeruli, renal blood flow, filtration fraction, and tubular transport (9, 16, 27, 30, 32), and quite possibly, immaturity of both glomerular and tubular function limits the ability of the younger animal to eliminate the nephrotoxin, independently of the renin angiotensin system.

Our conclusions with respect to assessment of the severity of renal failure are subject to certain limitations. Severe reduction in glomerular filtration rate (GFR) is the characteristic feature of acute renal failure, and direct and accurate measurements of inulin clearance were virtually impossible in this study because of

small size compounded by severe renal failure. BUN is a reflection of GFR providing that metabolic production rate of urea, the volume of distribution of urea, and urine flow rate are all constant. Normally, urea clearance varies between 20 and 65% of the GFR, depending upon urine flow rate (25). However, in the presence of renal failure, urea clearance might be expected to approximate inulin clearance much more closely in the functioning nephrons (10). Therefore, the observed BUN's are not explicable on the basis of urine flow. Similarly, it is improbable that 5- to 8-fold increases in BUN are the result of changes in the volume of distribution of urea. However, it is possible that urea production rates were altered to different degrees in the three groups by induction of acute renal failure. As can be seen in Figure 4, only the younger animals exhibited weight gains during the course of the study. Although both the 2- and the 4-week-old rats had similar patterns of weight gain, the courses of renal failure in these two groups, as assessed by BUN, were completely different. Moreover, it is precisely that group with the higher BUN which also had the more marked weight gain, thus rendering it most probably that differences in BUN reflect differences in glomerular filtration.

The reasons for higher mortality in the 2 week olds are incompletely understood. High mortality may be due to a lower tolerance to severe renal failure, as has been suggested also by survival rates of choline-deficient rats of different weights (22). Moreover, in our study, recovery of renal function was slowest in the youngest animals (Fig. 3), which might have been a contributory factor.

Interestingly, comparison of the 4 and 8 week olds reveals that the 4 week olds, despite having higher excretion rates of Na^+ and Cl^- before HgCl_2 injection, had a comparable degree of ARF. This contrasts with data obtained by us (5) and others (12, 28) in adult animals, showing that higher excretion rates are associated with milder renal failure. At least two explanations are possible. First, in younger animals, the responses to salt loading or blood volume expansion are different (3, 20, 34), and second, the protective effect of increased solute excretion may have threshold characteristics, and although Na^+ excretion was higher in 4 week olds, it was not as high as that observed in adult rats drinking 1% NaCl solution (4, 12, 28).

In summary, our results reveal important differences in both the components of the renin angiotensin system and the pattern of ARF during development in the rat. PRC declined with age, without concurrent changes in renal renin content. A pattern of delayed recovery of renal function was seen in the younger animals, probably attributable to a limitation in their ability to eliminate nephrotoxin because of immaturity of both glomerular and tubular function. PRC increased in the three groups, to different degrees, following HgCl_2 injection, but no simple correlation was observed between basal renal renin, plasma renin, the increase in plasma renin following HgCl_2 injection, and the severity of renal failure.

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