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Effect of Hypercapnic Acidosis on Renal Function in the Newborn Rabbit[1]

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ABSTRACT. Anaesthetized mechanically ventilated newborn rabbits were exposed to different degrees of hypercapnia. One hour of normocapnia was used as a control period. Renal function studies demonstrated an increase in renal vascular resistance with a concomitant decrease in effective renal plasma flow in all hypercapnic animals, combined with a less pronounced decrease in glomerular filtration rate. Filtration fraction rose significantly. A decrease in systemic blood pressure was only observed when the P_aCO_2 exceeded 100 mm Hg combined with an arterial pH below or equal to 7.10. We conclude that normoxemic hypercapnia in the newborn rabbit leads to an increase in renal vascular resistance and suggest that the renal vasoconstriction predominates at the level of the efferent arteriole. (*Pediatr Res* 20: 798–801, 1986)

Abbreviations

GFR, glomerular filtration rate
RBF, renal blood flow
RVR, renal vascular resistance
E_{PAH}, extraction of paraaminohippurate
C_{PAH}, clearance of PAH
MAP, mean arterial pressure
AVP, arginine vasopressin

A decrease in GFR and urine output has been described in newborn infants presenting with severe respiratory distress syndrome (1–3). The factors responsible for these disturbances include hypoxemia, hypercapnia, acidosis, and a fall in systemic blood pressure. The effect of isolated acute hypercapnic acidosis has not been extensively studied in animals whose nephrogenesis has not yet been completed. Rosenberg *et al.* (4) observed a consistent but not significant decrease in RBF in lambs undergoing mild hypercapnia (P_aCO_2 60–70 mm Hg). In contrast, no changes were observed in fetal or adult sheep. Alward *et al.* (5) observed a decrease in RBF, a stable GFR and an increase in RVR in piglets exposed to combined hypercapnia (P_aCO_2 70 mm Hg) and hypoxemia (P_aO_2 35 mm Hg). In adult animals most studies have been performed in anesthetized dogs, who frequently presented with a decrease in RBF and GFR when the P_aCO_2 exceeded 70 to 80 mm Hg (6–8). The purpose of the present study was to investigate the role of acute hypercapnia on renal function in the newborn rabbit before the end of nephrogenesis.

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MATERIALS AND METHODS

Experiments were performed on 5- to 12-day-old New Zealand White rabbits (n = 45), with a body weight varying from 81 to 214 g. The animals were anesthetized with 25 mg/kg sodium pentobarbital 0.5% intraperitoneally. Additional small doses of pentobarbital were administered when needed throughout the experiment. After tracheotomy the animals were artificially ventilated (Harvard 683 Rodent Ventilator, Millis, MA). The respiratory rate was kept constant at 40/min and tidal volume was adjusted for age and weight. Body temperature, recorded by an intraesophageal thermometer, was kept constant at 38.5° C, using a heating table and an infrared lamp. The femoral vein and artery were catheterized with polyethylene catheters (PE 10). Bladder catheterization was performed for urine sampling. Arterial and ventilatory pressures were continuously measured, using Statham transducers and recorded on a multichannel recorder (Model 7B Polygraph, Grass Instruments, Quincy, MA). The animals were paralyzed for the duration of the experiment with tubocurarine (25 μg/kg), which was repeated as needed. Following surgery the animals received a priming dose of an inulin-PAH solution (100 and 1.25 mg/kg, respectively). Thereafter a solution containing 50 g mannitol, 3 g inulin, 0.15 g PAH, 100 mmol NaCl and 5 mmol KCl/liter, was infused at a rate of 1 ml/100 g/h, to provide stable plasma levels of inulin (20–40 mg/100 ml) and PAH (0.2–1 mg/100 ml). $NaHCO_3$ was added to the solution in varying amounts (see below). The experiments were started 90–120 min after surgery when urinary flow and blood pressure had stabilized. During the experiment timed urinary collections of 30 min each were obtained and arterial blood sampling was performed at the midpoint of alternate urinary collection periods (Fig. 1). Clearances of inulin and PAH (C_{PAH}) were calculated from standard equations and used as estimates of GFR and effective renal plasma flow. The extraction of PAH was measured in a separate group of six hypercapnic newborn animals infused with 1 mmol $NaHCO_3$/kg per h [P_aCO_2 = 96 ± 6 (SEM) mm Hg for 60 min] and compared to the value previously observed in a group of 14 normocapnic newborn rabbits [P_aCO_2 = 40 ± 2 (SEM) mm Hg] studied in this laboratory (9). The extraction was 0.55 ± 0.03 (SEM) in the normocapnic animals and 0.56 ± 0.09 (SEM) in the hypercapnic animals. A value of 0.55 was subsequently used in the calculation of RBF, given by the formula (C_{PAH}/E_{PAH})/(1-hematocrit) and the filtration fraction (FF) as GFR/(C_{PAH}/E_{PAH}). RVR was calculated as MAP/RBF. The following chemical methods were used for blood and urine analysis: inulin and PAH by the Anthron-method and the Bratton-Marshall method, respectively (Technicon Autoanalyzer, Technicon Instruments Corporation, Terrytown, NY); gas-analysis under anaerobic conditions with a blood gas-ana-

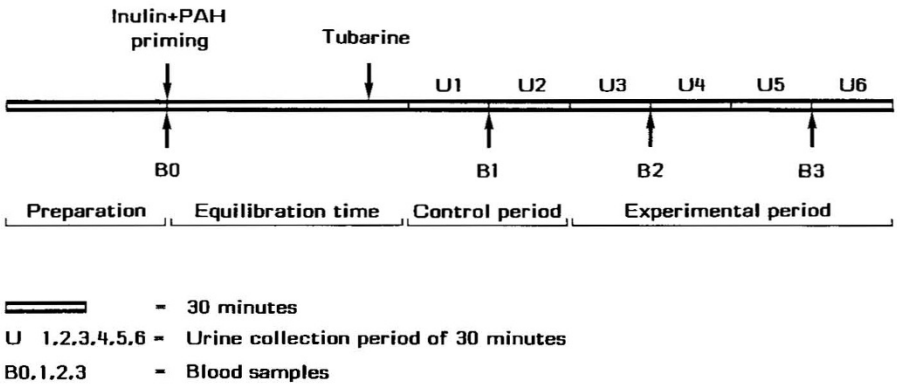

Fig. 1. Experimental protocol.

lyzer (pH/Blood–Gas Analyser 168, Corning, Halstead, Essex, England).

The following experimental protocols were used. The first two urinary collection periods always served as controls.

Group I (n = 8) and group II (n = 7). Normocapnia was maintained during 3 consecutive h; the infusion delivered 0.5 (group I) and 1 (group II) mmol $NaHCO_3$/kg/h, respectively.

Group III (n = 8) hypercapnia. A P_aCO_2 of 100 mm Hg was obtained using a fixed gas mixture containing 13% CO_2, 40% O_2, and 47% N_2. $NaHCO_3$ was added to the infusion to deliver 0.5 mmol $NaHCO_3$/kg/h. Hypercapnia was introduced following the first control hour and was maintained for 2 h.

Group IV (n = 8) hypercapnia. A P_aCO_2 of 100 mm Hg was similarly obtained and $NaHCO_3$ was added to the infusion to deliver 1 mmol $NaHCO_3$/kg/h. The protocol was the same as used in group I.

Group V (n = 8) hypercapnia. A P_aCO_2 of 80 mm Hg was obtained, using a fixed gas mixture containing 8% CO_2, 40% O_2, and 52% N_2. The infusion delivered 0.5 mmol $NaHCO_3$/kg/h.

Data analysis. Because of the large interindividual and interlitter variations each animal was used as his own control. The changes between control and experimental periods have been evaluated by calculating the significance of the difference between their means and zero, using the *t* test (10). In all cases a $p < 0.05$ was considered statistically significant.

RESULTS

Groups I and II. Infusion of 0.5 and 1 mmol sodium bicarbonate during 3 consecutive h of normocapnia did not significantly influence blood pH, P_aCO_2, and MAP. Except for a significant rise in urinary flow rate in group II due to the higher solute load, renal function remained essentially stable.

Effect of hypercapnia with a P_aCO_2 of 100 mm Hg and a $NaHCO_3$ infusion rate of 0.5 mmol $NaHCO_3$/kg/h (group III). The P_aCO_2 was increased from 39 to 103 mm Hg in the 1st h of hypercapnia and to 106 mm Hg in the 2nd h. The serum pH diminished abruptly from 7.48 to 7.10 during hypercapnia and remained stable for 2 h. MAP decreased slightly, but significantly, from 33.5 to 30.5 mm Hg, and urinary flow rate from 0.057 to 0.043 ml/min·kg ($p < 0.05$). There was no significant change in urinary output between the 1st and 2nd h of hypercapnia. C_{PAH} fell from 6.42 to 3.86 ml/min·kg within the 1st h of hypercapnia and to 3.33 ml/min·kg during the 2nd h. The clearance of inulin fell from 1.66 to 1.33 within 1 h and to 1.22 ml/min·kg (Table 1) within the 2nd h.

Effect of hypercapnia with a P_aCO_2 of 100 mm Hg and a $NaHCO_3$ infusion rate of 1 mmol $NaHCO_3$/kg/h (group IV). The $NaHCO_3$ infusion rate was doubled in this group to partly blunt the decrease in serum pH while maintaining the same P_aCO_2. The increase in P_aCO_2 was similar to that present in the first group, from 39 to 104 mm Hg. The decrease in serum pH was less pronounced, from 7.51 to 7.17. No decrease in MAP was observed and although urinary flow rate decreased from 0.064 to 0.058 ml/kg·min, the fall was not significant. However, the decrease of C_{PAH} was similar from 5.91 to 4.14 in the 1st hypercapnic h and to 3.07 ml/kg·min in the 2nd hypercapnic h. The decrease in GFR observed during the 1st h of hypercapnia was not significant from 1.56 to 1.36 ml/kg·min, but later reached statistical significance ($p < 0.05$) (Table 1).

Effect of hypercapnia with a P_aCO_2 of 80 mm Hg and a $NaHCO_3$ infusion rate of 0.5 mmol $NaHCO_3$/kg/h (group V). The P_aCO_2 increased from 35 to 78 mm Hg in the 1st h and to 80 mm Hg in the 2nd h of hypercapnia. Serum pH decreased from 7.51 to 7.20 and remained stable afterward. MAP remained stable throughout the experiment and the observed decrease in urinary volume was not significant. A marked decrease in C_{PAH} from 7.45 to 5.16 during the 1st h and to 4.43 ml/kg·min during the 2nd h, was also observed in this group. GFR declined from 2.03 to 1.57 and to 1.64 ml/kg·min in the 1st and 2nd h, respectively (Table 1). The decrease in RBF is shown in Table 2 together with the concomitant increase in RVR. Filtration fraction increased in all hypercapnic groups.

DISCUSSION

The present study demonstrates that acute hypercapnia in the anesthetized newborn rabbit is associated with major changes in renal function. Three different experimental protocols were used in order to establish this. The significant decrease in MAP in group III could be prevented by adding 0.5 mmol $NaHCO_3$/kg/h to the infusion in group IV. This may be due to the higher level of serum pH or to the expansion of extracellular volume by the hyperosmolar infusion. The effect of a fall in MAP was thus excluded in this group and was also absent in the other groups. A P_aCO_2 of 100 mm Hg with a serum pH of 7.15–7.17 (group IV) led to nearly the same changes as a P_aCO_2 of 80 mm Hg and a serum pH of 7.20 (group V). Only the GFR in group V seemed to stabilize. The observed effects can be due to the hypercapnia, the acidosis, or to the combination of these factors, but remained present in all experiments thus supporting the consistency of the data. The most striking change is the decline in PAH clearance observed in all hypercapnic groups. This could reflect a true decrease in RBF or a decrease in PAH extraction in the hypercapnic animals. To exclude this second possibility, additional experiments were performed in newborn animals of the same age, undergoing hypercapnia for 60 min. The PAH extraction values were comparable to those observed in normocapnic animals, thus demonstrating that hypercapnia does not change PAH extraction, and that the drop in C_{PAH} corresponds to a true decrease in RBF. A 10% decrease in PAH extraction was observed by Anderson *et al.* (11, 12) during hypercapnic acidosis in dogs, but was apparently not found in other studies of the same group (13, 14), nor was any change in PAH extraction

Table 1. *Effect of hypercapnia on blood pH, MAP, and renal function**

		P_{aCO_2} (mm Hg)			Blood pH			MAP (mm Hg)			V (ml/min·kg)			C_{PAH} (ml/min·kg)			C_{inulin} (ml/min·kg)		
		C	1	2	C	1	2	C	1	2	C	1	2	C	1	2	C	1	2
I	Mean	42	42	38	7.48	7.45	7.49	31.3	30.3	29.6	0.071	0.075	0.081	5.32	4.79	4.70	1.71	1.55	1.55
	SEM	2.4	2.0	1.7	0.03	0.03	0.02	1.2	1.4	1.7	0.008	0.010	0.015	0.87	0.48	0.64	0.26	0.22	0.27
$n=8$	p																		
II	Mean	37	37	36	7.51	7.52	7.52	35.5	34.5	35.0	0.054	0.064	0.079	6.58	6.88	6.43	1.68	1.69	1.74
	SEM	2.3	1.7	1.6	0.03	0.02	0.02	2.0	2.0	1.8	0.009	0.008	0.009	1.32	1.54	1.65	0.18	0.23	0.14
$n=7$	p											†							
III	Mean	39	103	106	7.48	7.10	7.10	33.5	30.5	29.5	0.057	0.043	0.050	6.42	3.86	3.33	1.66	1.33	1.22
	SEM	2.4	1.3	1.8	0.01	0.01	0.01	1.0	1.3	1.3	0.007	0.005	0.009	0.67	0.45	0.61	0.14	0.14	0.24
$n=8$	p		‡	‡		‡	‡		†	†		†			§	§		†	
IV	Mean	39	101	104	7.51	7.15	7.17	32.8	33.9	34.7	0.064	0.058	0.060	5.91	4.14	3.07	1.56	1.36	1.29
	SEM	1.7	1.5	3.2	0.02	0.01	0.01	1.2	1.8	2.0	0.006	0.005	0.003	0.39	0.30	0.34	0.12	0.07	0.11
$n=8$	p		‡	‡		‡	‡, ‖								†	‡, ¶			†
V	Mean	35	78	80	7.51	7.20	7.21	33.7	32.6	32.9	0.079	0.056	0.070	7.45	5.16	4.43	2.03	1.57	1.64
	SEM	2.0	1.9	1.8	0.02	0.02	0.02	0.7	0.8	1.1	0.011	0.004	0.007	0.43	0.38	0.48	0.20	0.14	0.19
$n=8$	p		‡	‡		‡	‡								§	§		†	

* V, urine flow rate: C_{inulin}, glomerular filtration rate; C, control period; 1, first period of hypercapnia; 2, second period of hypercapnia.
† Significant *vs* control <0.05.
‡ Significant *vs* control <0.001.
§ Significant *vs* control <0.01.
‖ Significant *vs* first period <0.05.
¶ Significant *vs* first period <0.001.

Table 2. *Values of RBF, RVR, and FF before and during hypercapnic acidosis**

		RBF (ml/min·kg)			RVR (mm Hg/ml/min·kg)			FF (%)		
		C	1	2	C	1	2	C	1	2
I	Mean	14.06	12.38	11.95	2.52	2.55	2.77	18.2	17.5	17.4
	SEM	2.30	1.21	1.64	0.31	0.18	0.38	1.0	1.0	0.9
$n=8$	p									
II	Mean	17.14	17.29	15.92	2.61	2.49	2.83	17.2	16.0	18.4
	SEM	3.36	3.68	3.93	0.50	0.44	0.46	2.7	2.4	2.4
$n=7$	p									
III	Mean	15.63	9.69	8.21	2.20	3.36	4.57	15.3	18.4	19.7
	SEM	1.12	1.14	1.51	0.26	0.41	0.92	1.3	1.7	0.8
$n=8$	p		§	§		†	†		†	§, ‖
IV	Mean	15.40	10.63	7.99	2.17	3.29	4.70	14.6	18.3	23.7
	SEM	1.11	0.75	0.97	0.14	0.33	0.55	0.7	0.9	1.5
$n=8$	p		§	‡, §		†	§, ‖		§	‡, **
V	Mean	19.36	13.20	11.53	1.76	2.54	3.12	14.8	17.0	20.4
	SEM	0.99	0.83	1.33	0.08	0.17	0.34	0.9	1.2	0.7
$n=8$	p		‡	‡		†	§		†	‡, ¶

* FF, filtration fraction: C, control period: 1, first period of hypercapnia: 2, second period of hypercapnia. A constant extraction factor of PAH of 55% was used for the calculation of RBF.
† Significant *vs* control <0.05.
‡ Significant *vs* control <0.001.
§ Significant *vs* first period <0.01.
‖ Significant *vs* control <0.05.
¶ Significant *vs* first period <0.01.
** Significant *vs* first period <0.001.

during hypercapnia mentioned by Norman *et al.* (7) and by Berns *et al.* (15). This is in agreement with studies using either a sine wave electromagnetic flowmeter (6) or microspheres (4, 5) for measuring RBF.

The increase in filtration fraction suggests that the vasoconstriction induced by hypercapnia, predominates at the level of the efferent arteriole. The effects observed in our experiments are similar to the changes described in the adult dog by several

authors (6–8). Rose *et al.* (14) discussed the validity of these results in dogs, arguing that the introduction of artificial ventilation and the use of pentobarbital could be responsible for the observed decrease in RBF, which they did not observe in their experiments on conscious dogs. Indeed Walker *et al.* (16) have clearly shown that pentobarbital can depress both RBF and GFR. However, in the protocol used by Rose, the levels of P_aCO_2 were much lower than in our study, or in the studies mentioned above (6–8). It should also be noted that in another study the same group did not find a decrease in RBF in similar conditions despite the use of pentobarbital, at least when MAP was stable (13). That the decrease in RBF observed in our experiments is not due either to pentobarbital or to the artificial ventilation is demonstrated by the stability of the two control groups throughout the experiment. Several mechanisms may contribute to the decrease in RBF observed during hypercapnia. A major activation of the renin-angiotensin system has been found in neonates with respiratory distress syndrome (17) as well as in adults with acute hypercapnia (18). This has been confirmed in animal experiments by Kurtz and Zehr (19). A predominant effect of angiotensin II on the efferent arteriole of the rabbit kidney has been observed by Edwards (20), which may explain the increase in filtration fraction in our experiments.

The changes in RBF observed in our experiments can thus be due to a direct stimulation of the renin-angiotensin system by hypercapnia, or to a stimulation via the renal nerves as suggested by different authors (7, 12, 13, 15, 20).

AVP may be another important factor. An hyperosmolar infusion was used in our newborn rabbits. This may have stimulated AVP secretion, as has been demonstrated in the fetal sheep (21). However, the hypersecretion of AVP did certainly not influence renal hemodynamics or urinary volume in the two control groups. Hypercapnia per se also stimulates AVP secretion, as observed in the adult dog by Berns *et al.* (15). Thus a role for AVP in our experiments cannot be excluded.

In conclusion, acute hypercapnic acidosis in the anesthetized newborn rabbit leads to an increase in RVR, a decrease in RBF and, as a result of this, a decrease in GFR. We suggest that the renal disturbances observed in neonates with respiratory distress syndrome could be due, at least in part, to the effect of hypercapnic acidosis superimposed on the already known effects of hypoxemia.

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