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Thirst in the Rat After Ligation of the Inferior Vena Cava: Role of Angiotensin II

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MANN, J. F. E., A. K. JOHNSON, W. RASCHER, J. GENEST AND D. GANTEN. *Thirst in the rat after ligation of the inferior vena cava: Role of angiotensin II.* PHARMAC. BIOCHEM. BEHAV. 15(3) 337–341, 1981.—The role of angiotensin II in thirst states after ligation of the inferior vena cava above (CLA) or below (CLB) the origin of the renal veins as compared to sham operated controls was evaluated 24 hrs after ligation. Water intake was enhanced in CLB rats and even more so in CLA rats. Plasma angiotensin II and urea concentrations and serum osmolality were increased in CLA rats. Plasma sodium concentration and hematocrit were reduced in CLA rats, and hematocrit in CLB rats as well. Water intake in CLA rats was retarded by IV infusions of saralasin. Saralasin infusions in CLA rats resulted in a dramatic increase of plasma angiotensin II concentrations. Ligation of the inferior vena cava induces major changes in body fluid homeostasis, which are more pronounced in CLA than in CLB rats. The increase in water intake in CLA rats appears to be partly mediated by angiotensin II.

Angiotensin Angiotensin antagonist Renin Thirst Vena cava ligation

EVIDENCE indicates that multiple humoral and neural mechanisms mediate the thirst state which accompanies dehydration [2, 5, 8]. The renin-angiotensin-system has been proposed to be one of the factors which stimulate drinking behavior and thus maintain the stability of the "milieu intérieur." In several animal models of thirst, nephrectomy will diminish or abolish water intake [8]. Also, in man, there is some evidence that a renal-related mechanism may be dipsogenic, for it has been reported that excessive thirst in patients with high renin levels is relieved by bilateral nephrectomy [4,19].

Ligation of the inferior vena cava has been a useful model for the study of thirst, since this manipulation simulates a deficit in blood volume via a reduction of venous return to the heart. Several lines of evidence have evolved to support a dipsogenic role for the renin-angiotensin-system in caval ligation-induced thirst. These include the fact that this form of experimental thirst does not occur after nephrectomy [7], and that the manipulation raises plasma renin concentrations [14] and angiotensin II levels [13]. However, previous results by Rolls and Woods [20] appear to be inconsistent with the hypothesis that the renin-angiotensin-system mediates thirst following caval ligation. These investigators found that intravenous (IV) infusions of saralasin, a competitive antagonist of angiotensin II, did not attenuate water intake. Two explanations were proposed to account for the failure of saralasin to attenuate thirst following caval ligation: (1) that angiotensin is not normally involved in the mediation of this form of experimentally-induced drinking, and/or (2) that there is a redundancy in the control of drinking which can compensate for a blocked mechanism (e.g. volume or baroreceptor mechanisms) [20]. However, a third hypothesis not considered by these investigators is that the endogenous renin-angiotensin-system has a substantial residual capacity and, in the face of a hypotensive-hypovolemic stimulus, can overcome the peripheral blockade by increased renin release and subsequent angiotensin II generation [9].

The present work was conducted to provide thorough examination of the role of the renin-angiotensin-system in caval ligation-induced thirst. In particular, we have examined the capability of this humoral system to respond in the face of systemic application of saralasin. Also, the concomitant changes in plasma electrolytes and osmolality following caval ligation were examined. The vena cava was ligated above (CLA) and below (CLB) the origin of the renal veins since both procedures have been employed [7, 13, 14, 20] but their differential effect on body fluid homeostasis is not completely understood.

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METHOD

Rats were maintained in single cages in a room with constant temperature (24±2°C) and a 12 hr dark/light cycle. Tap water and a regular rat chow were offered ad lib prior to the experiments.

Experiment A. Comparisons of Water Intake and Blood Parameters Between CLA and CLB Rats

Different anatomical locations of the inferior vena cava ligation were compared as to their effects on water intake, plasma angiotensin II, and other body fluid parameters relevant for drinking behavior.

Male albino rats (335–370 g body weight; Thomae, Lörrach, GFR) were anesthesized with ether and their abdomen shaved. The abdominal cavity was opened, the intestines were gently retracted and covered by a gauze pad, soaked with warmed 0.9% saline. The inferior vena cava was dissected free and completely ligated shortly above (CLA) (n=8) or below (CLB) (n=8) the origin of the renal veins. In sham operated animals (n=8) a short piece of silk thread was placed under the vena cava but not constricted. The abdominal wall was closed in layers and the animals were returned to their cages. Two hours later, water was offered to the rats and water intake was measured by weighing the plastic bottles 2½, 3, 4, 5, 6, and 24 hrs after ligation. When bottles were inverted and placed on the cage, a maximum of 0.1 ml of water was lost. No food was present during that time. Twenty-three to 25 hrs after initial surgery, the rats were anesthesized with ether, the abdominal wall was opened and blood was withdrawn from the aorta [13,16]. The first two ml of blood were collected for plasma angiotensin II measurement into a syringe containing 0.1 ml of a solution of 125 mM ethylenediaminetetraacetic acid (EDTA), and 26 mM 1–10 orthophenanthroline as enzyme inhibitors. Time interval from the beginning of anesthesia until the end of the 2 ml blood collection never exceeded 120 sec. Additional blood samples were taken for determination of plasma concentrations of sodium and urea, of serum osmolality and of hematocrit. The blood was immediately placed on ice and centrifuged at 4°C at 10,000 g for 10 min. Plasma was separated and stored frozen at −20°C, and serum at 4°C.

Experiment B. Effects of Cumulative Saralasin Infusions on Thirst in CLA Rats

Cumulatively increasing doses of IV saralasin had previously been found to produce no agonistic pressor effect [15]. Saralasin was applied in this manner in CLA rats which exhibited higher plasma angiotensin II levels than CLB rats.

In male albino rats (270–310 g, Canadian Breeding Farm, St. Constant, Quebec, Canada) the inferior vena cava was completely ligated above the origin of the renal veins as described, under light ether anesthesia. A catheter (PE 50) was inserted into the right jugular vein and brought under the skin to exit through the scruff of the neck. The rats were returned to their cages and 35 min after ligation, an IV infusion was started. (sar^1, ala^8)-Angiotensin II (Peninsula, San Carlos, CA) diluted in 0.9% saline (n=9) was infused at cumulative doses of 0.7, 1.7, 3.7, and 8.5 μg/kg/min for 25 min at each dose and at 20 μg/kg/min for an additional 75 min. In previous experiments it was found (unpublished observations) that 20 μg/kg/min of saralasin will produce saralasin plasma concentrations of about 100 pmol/ml (measured by radioimmunoassay [11]), enough to antagonize 6 pmol angiotensin II/ml, the highest plasma level measured in CLA rats (see Fig. 2). In control rats (n=10), 0.9% saline was infused in the same volumes as saralasin (i.e. 0.2, 0.5, 1.1, and 2.5 μl/min for 25 min each, and 6 μl/min for 75 min). Forty-five min after start of the highest infusion rate all rats received their water bottles (no food was present). Water intake was measured at 15, 30, and 60 min after receiving water.

Experiment C. Effect of Saralasin Infusions on Plasma Angiotensin II Levels in CLA Rats

Single doses of saralasin up to 50 μg/kg/min did not reduce water intake of CLA rats in a previous report [20]. The capacity of the renin-angiotensin-system to override the antagonistic action of saralasin was evaluated.

In male albino rats (220–280 g, Thomae, Lörrach, GFR) the inferior vena cava was completely ligated above the origin of the renal veins (n=10) or sham ligated (n=12) and catheters were inserted into the jugular vein as described above. An IV infusion lasting 30 min was started 165 min after surgery at a rate of 0.1 ml/kg/min. Half of the rats of each group received 0.9% saline, the other half saralasin at 50 μg/kg/min, as described by Rolls and Wood [20]. At the end of the 30 min infusion period, the rats were rapidly anesthesized with ether and blood was withdrawn from the abdominal aorta (see above) for determination of plasma angiotensin II concentration. Food and water were not offered during this experiment.

Variables Measured

Plasma sodium concentration was measured by flame photometry; plasma urea by the urease method, the amount of ammonium carbonate being formed was quantified photometrically. Serum osmolality was determined by freezing point depression and hematocrit by the use of microcapillary tubes. Plasma concentration of angiotensin II was evaluated by radioimmunoassay as described [16]. Cross reactivity of the angiotensin II antibody with other peptides was as follows: angiotensin I, 1%; angiotensin II (2–8) heptapeptide, (3–8) hexapeptide, and (4–8) pentapeptide, 100%; (sar^1, ala^8)-angiotensin II, less than 0.001%; substance P, bradykinin, less than 0.01%.

Values are given as mean±SEM. Data were analyzed for significance of differences by analysis of variance (ANOVA) followed by Scheffé test [21].

RESULTS

Ligation of the inferior vena cava resulted in an effective stimulation of water intake (Table 1). CLA rats drank more than controls at 3 hrs and at every point in time thereafter when water intake was measured cumulatively. In CLB rats, water intake was found to be higher than in controls 4 and 5 hrs after surgery. There were major changes in CLA rats in all blood parameters measured (Table 2). Angiotensin II and urea plasma concentrations were about 5.5 and 10 fold higher, respectively, than in controls. In addition, we observed a higher osmolality, a lower hematocrit and a lower plasma sodium level in CLA rats than in controls. In CLB rats, hematocrit was found to be lower than in controls (Table 2).

Water intake was partly inhibited in CLA rats by prolonged infusion of saralasin, a competitive angiotensin II analog (Fig. 1). Thirty min after the end of the infusion

TABLE 1

CUMULATIVE WATER INTAKE IN CLA, CLB, AND SHAM OPERATED RATS AT DIFFERENT TIME INTERVALS FOLLOWING SURGERY

	Time (hrs)					
	2.5	3	4	5	6	24
Water intake (ml)						
CLA	2.25 ±0.27	3.13* ±0.31	4.50† ±0.64	5.88† ±0.72	7.19† ±0.84	15.06*‡ ±1.13
CLB	1.81 ±0.28	2.94 ±0.45	4.69† ±0.74	4.94* ±0.85	5.69 ±0.97	11.31 ±1.34
Controls	1.63 ±0.44	2.00 ±0.19	2.25 ±0.25	3.01 ±0.19	3.63 ±0.26	9.19 ±1.02

Values are means ± SEM; n=8 per group.
*$p<0.05$; †$p<0.01$ as compared to controls; ‡$p<0.05$ CLA compared to CLB.

TABLE 2

MEASUREMENT OF VARIOUS PHYSIOLOGICAL PARAMETERS IN RATS 1 DAY AFTER LIGATION OF THE INFERIOR VENA CAVA ABOVE (CLA) AND BELOW (CLB) THE ORIGIN OF THE RENAL VEINS AND IN SHAM OPERATED CONTROLS

	Plasma angiotensin II concentration (fmol/ml)	Plasma sodium concentration (mEq/l)	Plasma urea concentration (mmol/l)	Serum osmolality (mosmol/kg)	Hematocrit (%)
CLA	639.6†§ ±103.1	126.2†‡ ± 1.3	59.9†§ ± 4.0	341.5†§ ± 5.5	38.2† ± 1.1
CLB	140.8 ± 19.2	130.5 ± 1.1	6.7 ± 0.5	296.6 ± 1.7	41.0† ± 0.8
Controls	117.4 ± 12.8	133.0 ± 0.2	5.5 ± 0.2	294.6 ± 1.3	45.1 ± 0.2

Values are means ± SEM; n=8 per group.
*$p<0.05$; †$p<0.001$ compared to controls.
‡$p<0.05$; §$p<0.001$ CLA compared to CLB.

period, there was no longer a difference between saline- and saralasin-treated CLA rats.

Water intake in saline infused CLA rats (Experiment B, Fig. 1) 4 hrs after ligation was comparable to CLA rats in Experiment A (Table 1, 5.23±0.75 vs 4.5±0.64 ml, respectively). In saralasin infused CLA rats (Experiment B, Fig. 1) water intake at the end of the infusion period (3.5 hrs after ligation) was similar to sham operated controls of Experiment A at 4 hrs (Table 1, 1.82±0.85 vs 2.25±0.25 ml, respectively).

Plasma angiotensin II concentrations were higher in CLA rats as compared to sham operated controls (205 min after surgery), whether both were infused with saline ($p<0.01$) or saralasin ($p<0.01$) (Experiment C, Fig. 2). Saralasin infusions elicited a marked rise in plasma angiotensin II levels in CLA rats ($p<0.01$) as compared to CLA rats receiving saline infusions or as compared to sham operated controls receiving either saralasin or saline (Fig. 2).

DISCUSSION

The present series of studies extends the characterization of effects of caval ligation on body fluid homeostasis and lends further support to the conclusion that the renin-angiotensin-system makes a significant contribution to the drinking following this experimental manipulation. The initial study confirms that constriction of the vena cava either above or below the renal vein produces significant drinking in water replete rats. Comparable water intake 6 hrs following CLA and CLB was reported [7]. In our hands, CLA appeared to be slightly more effective than CLB since there were significant elevations in intake over control levels beginning at 3 hrs until the end of 24 hr test period in CLA rats only. It is of note, however, that water intake was measured cumulatively (Table 1). When water intake overnight was analyzed separately (6 to 24 hrs), there was no significant difference between CLA and control rats (see Table 1). This

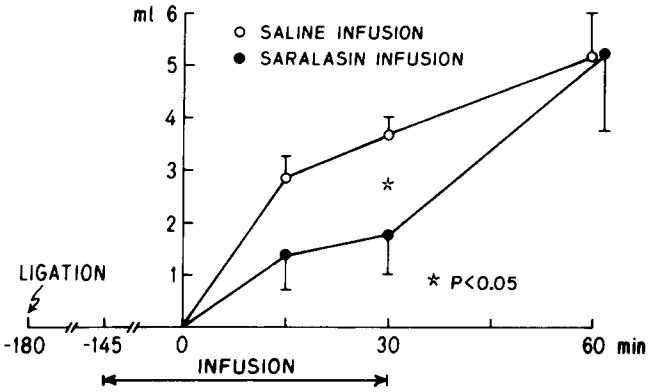

FIG. 1. Water intake in rats with ligation of the vena cava inferior above the renal veins. Water access was given 180 min after surgery and 145 min after start of infusion with cumulative doses of saralasin (●, n=9), or with 0.9% saline (○, n=10, Experiment B). The ordinate gives water intake in ml. The asterisk indicates statistical significance (ANOVA). Values are means±SEM.

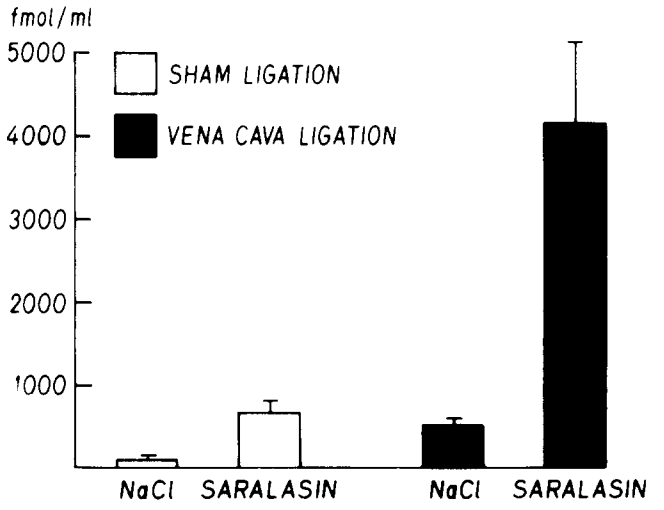

FIG. 2. Plasma angiotensin II concentrations in rats with ligation of the inferior vena cava above the renal veins (n=10), and in controls (n=12), 195 min after surgery. Half of the rats of each group received 50 μg/kg/min of saralasin IV for 30 min before sacrifice [20], the other half 0.9% saline IV (Experiment C). The ordinate gives plasma angiotensin II levels in fmol/ml. For details see Results. Values are means±SEM.

has also been reported by others [14]. Thus, the major stimulation of thirst following caval ligation occurred during the first hrs after surgery.

The blood parameter data collected 24 hrs after ligation in Experiment A indicated that there were significant elevations in plasma osmolality, angiotensin II levels, and urea concentrations in CLA rats. It is likely that the plasma angiotensin II levels were elevated sufficiently to contribute to water intake throughout the 24 hrs period. In previous studies we have determined the levels of circulating angiotensin II at threshold dipsogenic dose [12] to be approximately 200 fmol/ml plasma [16]. In addition, there may be a contribution to thirst by factors arising as a manifestation of impaired renal function in rats with ligation above the renal veins. It has been demonstrated [3] that nephrectomized drink more than intact animals under ad lib conditions over 24 hrs.

The water intake results are in agreement with others [7,14], and indicate that despite elevated plasma angiotensin levels in CLA rats at 24 hrs, drinking behavior may not be significantly elevated. It therefore appears that a factor(s) may inhibit drinking in CLA rats. One such factor may be an expansion of the intravascular volume as a consequence of the enhanced water intake associated with oliguria [7]. Evidence for this are the striking decrease of hematocrit and of plasma sodium concentration. These changes may activate inhibitory processes similar to those that inhibit vasopressin secretion [6,10]. Central receptors may be inhibited by hyponatremia despite the hyperosmolality since the increase in osmolality can be totally attributed to the rise of plasma urea. This may not be an effective thirst stimulus because it readily equilibrates with the intracellular space. Furthermore, the hyponatremia may counteract inputs from left atrium receptors which are probably still sensing a volume deficit at 24 hrs.

Experiment B indicated that saralasin treatment may reduce water intake in caval ligated rats. This reduction in the rate of water intake would be expected if the competitive blocker was attenuating the dipsogenic action of angiotensin II. Saralasin was infused by slowly incrementing the dose. This graded method of delivery has been employed in normal rats and man to minimize the agonistic pressor action of the antagonist [11,15]. A rise of blood pressure upon injection of a single bolus dose of saralasin is typically observed [18]. Thus, the failure [20] to report an initial suppression of drinking in CLA rats treated with saralasin may have been due to the fact that the antagonist was delivered at a fixed dose rather than in a graded manner, as applied in the present study. In Experiment B, no sham operated controls were included. It is of note, however, that water intake in saralasin infused CLA animals dropped to levels observed in sham operated controls of Experiment A.

Most likely saralasin led to a reduction of blood pressure in vena cava ligated rats [22]. This may interfere with the physical ability of the animals to drink [22]. On the other hand, hypotension may be a further stimulus for water intake. It should be noted that fluid consumption was not totally absent but rather suppressed in saralasin infused CLA rats, indicating that they were still able to drink.

Experiment C demonstrates a reserve capacity of the renin-angiotensin-system to generate large quantities of angiotensin II which may counteract the antagonistic effect of saralasin. In previous experiments, we have examined [16] various types of challenges which are capable of activating the renin-angiotensin-system. In those studies a mean plasma concentration of angiotensin II of approximately 1600 fmol/ml was seen in anesthetized rats with malignant renal hypertension [16]. These levels of endogenously generated peptide were the greatest produced by any experimental treatment in this laboratory up until this time. Angiotensin II levels approximately three times as high were achieved in the presence of saralasin in CLA rats in the present study. Because angiotensin was measured by radioimmunoassay it might be questioned whether these high levels of plasma angiotensin II were due to cross-reactivity of the angiotensin

antibody with saralasin. However, this is unlikely because in sham operated controls receiving the same amount of saralasin, much lower plasma concentrations of angiotensin II were measured. Thus, cross-reactivity of saralasin with the angiotensin II antibody (less than 0.001%) does not appear to be responsible for the high levels of plasma angiotensin II.

It would be necessary to infuse large amounts of saralasin to block the action of angiotensin II when this peptide is circulating at levels in the order of 2 to 6 pmol/ml plasma. When plasma saralasin was measured by a specific radioimmunoassay, it was found that a 20 μg/kg/min infusion produced circulating levels of about 100 pmol saralasin/ml plasma ([11], and unpublished observations). If plasma ratios of antagonist to agonist of 10:1 are required for blockade of the action of angiotensin II [17], then, based on the present results, it appears that the endogenous renin-angiotensin-system would have the capacity to ultimately override infusions of saralasin below 10 μg/kg/min. Therefore, 20 μg/kg/min were given in Experiment B in cumulative fashion to reach effective antagonitic concentrations of saralasin but to avoid too high doses which may be agonistic, especially as bolus.

In summary, the present work provides additional evidence that ligation of the vena cava induces major changes in body fluid homeostasis that lead to thirst and volume expansion. The observed changes of fluid retention are less prominent when the vena cava is ligated below the renal veins as compared to ligation above this level. Furthermore, considering the reserve capacity of the renin-angiotensin-system, it is likely that the increase in fluid intake is partly mediated by angiotensin II. Inhibition of drinking by saralasin in rats with the vena cava ligated may not be easily feasible because of the potential of the renin-angiotensin-system to overcome the competitive blockade when doses of the inhibitor are too small. On the other hand, high doses of saralasin given at a fixed rate may exert an agonistic effect on drinking. This hypothesis will have to be tested in further experiments.

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