The effect of small doses of antidiuretic hormone on renal excretion of sodium and water in saline loaded acutely hypophysectomized rats

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Summary. Small doses of vasopressin (200 μU min^{-1} kg^{-1}) did not influence the loss of ability of acutely hypophysectomized rats to react by natriuresis to the extracellular fluid volume expansion with saline, inspite of restoring their arterial blood pressure to normal. It is concluded that the level of antidiuretic hormone efficient enough to concentrate urine is not a decisive factor in the homeostatic mechanism promoting the 'volume' natriuresis.

It has been shown in our previous work[2] that small doses of antidiuretic hormone (ADH) did not influence the natriuretic response of normal rats to the extracellular fluid volume (ECFV) expansion with isotonic saline infusion (the so-called 'volume' natriuresis). Only the 'volume' polyuria was diminished due to the increased reabsorption of free water. To analyze the role of ADH in the renal regulation of the ECFV further, we examined the effect of ADH in experiments on acutely hypophysectomized rats. These animals failed to respond by natriuresis to saline loading; however, they increased effectively free water excretion[3,4].

Material and methods. 23 male Wistar rats (Velaz, Prague) weighing 240–290 g were anaesthetized by i.p. administration of Inactin (Promonta) 100 mg kg^{-1} b.wt. Surgical preparation comprised tracheotomy and cannulations of carotid artery, jugular vein, femoral artery and vein and urinary bladder with polyethylene catheters. In 16 rats,

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The influence of ADH on the blood pressure (BP), inulin clearance (C_{in}), sodium excretion ($U_{Na}V$), urine output (V) and clearance of osmotically free water (C_{H_2O}) in acutely hypophysectomized rats (Hypox + ADH, group 3) compared to hypophysectomized rats without ADH infusion (Hypox, group 2). For reference the same parameters in normal rats are presented, too (group 1)

Time (min)	Period	1 Normal rats (n = 7)	2 Hypox (n = 10)	3 Hypox + ADH (n = 6)	p 1:2	2:3
			BP torr			
0– 20	1	136.6 ± 6.2	116.3 ± 4.1	131.3 ± 7.5	A	ns
20– 40 Inf.	2	138.3 ± 6.6	116.2 ± 1.8	138.7 ± 6.2	B	B
40– 60	3	132.6 ± 5.7	112.5 ± 3.2	137.0 ± 6.1	B	B
60– 80	4	132.4 ± 5.7	110.3 ± 3.2	136.0 ± 6.6	B	B
80–100	5	130.1 ± 5.8	103.3 ± 4.6a	128.0 ± 9.8	B	A
			Cin ml min^{-1}			
0– 20	1	1.15 ± 0.12	0.81 ± 0.09	0.95 ± 0.18	A	ns
20– 40 Inf.	2					
40– 60	3	1.74 ± 0.17a	1.42 ± 0.17b	1.11 ± 0.15	ns	ns
60– 80	4	1.60 ± 0.14a	1.34 ± 0.19a	0.84 ± 0.11	ns	ns
80–100	5	1.56 ± 0.27	1.07 ± 0.12	1.02 ± 0.23	ns	ns
			$U_{Na}V$ μmoles min^{-1}			
0– 20	1	0.09 ± 0.02	0.06 ± 0.01	0.07 ± 0.03	ns	ns
20– 40 Inf.	2	5.67 ± 1.70b	0.19 ± 0.04b	0.35 ± 0.10a	B	ns
40– 60	3	4.45 ± 0.74c	0.22 ± 0.06a	0.67 ± 0.22a	C	ns
60– 80	4	2.93 ± 0.64c	0.15 ± 0.03b	0.36 ± 0.10a	C	ns
80–100	5	2.37 ± 0.49c	0.10 ± 0.02	0.41 ± 0.24c	C	C
			V μl min^{-1}			
0– 20	1	6.5 ± 2.3	11.4 ± 1.6	2.8 ± 0.6	ns	C
20– 40 Inf.	2	80.5 ± 19.3b	44.4 ± 5.4c	8.4 ± 1.1b	ns	C
40– 60	3	50.6 ± 5.3c	61.8 ± 8.6c	9.0 ± 1.5b	ns	C
60– 80	4	31.1 ± 3.6c	52.7 ± 6.5c	8.1 ± 2.0a	A	C
80–100	5	21.2 ± 3.3b	45.2 ± 5.3c	6.6 ± 1.7	B	C
			C_{H_2O} μl min^{-1}			
0– 20	1	−16.6 ± 2.8	−2.4 ± 1.4	−12.4 ± 3.3	C	A
20– 40 Inf.	2	−13.2 ± 16.5	22.3 ± 6.5	−20.7 ± 5.9	A	C
40– 60	3	−14.9 ± 6.7	43.5 ± 8.6	−21.3 ± 3.5	C	C
60– 80	4	−22.2 ± 3.6	36.8 ± 6.7	−16.1 ± 3.6	C	C
80–100	5	−22.8 ± 2.5	32.1 ± 5.8	−15.4 ± 4.8	C	C

All excretory values are calculated per g of kidney wt and presented as means ± SE. Inf., period of isotonic saline infusion. p, statistical significance of a difference between corresponding periods in intergroup statistical analysis: A, $p < 0.05$; B, $p < 0.01$; C, $p < 0.001$, ns = not significant ($p > 0.05$). Statistical significance of values in periods 2–5 compared to the first (control) period in each respective group is indicated as: a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$.

hypophysectomy was performed through the parapharyngeal approach. Following the operation, a continuous infusion of inulin-^{14}C and lysine-vasopressin (Sandoz) 200 $\mu U\ min^{-1}\ kg^{-1}$ was started in 6 hypophysectomized rats. The other 10 hypophysectomized and 7 normal non-hypophysectomized rats were given only the inulin-^{14}C infusion. After a 1-h equilibration period, a few minutes before starting the first 20-min urine-sampling period, each animal received 200 U heparin i.v. Intravenous infusion of isotonic saline in the amount of 4% of each animal's b.wt was completed in the next 20-min-interval. Another 3 urine samples were taken in the subsequent 60-min-duration of the experiment. Blood samples (0.8 ml) for analyses were withdrawn from the carotid artery in the middle of the first, third and at the end of the fifth period after the cannulas were flushed out by means of an arteriovenous shunt. The volume of blood withdrawn was replaced immediately by the same volume of fresh heparinized rat blood. Arterial blood pressure was recorded continuously by a polygraph. The results were statistically evaluated by means of the Student t-test with correction of t-criterion when the F-values were significant[5,6].

Results and discussion. The results are summarized in the table. The infusion of saline increased significantly sodium and urine excretion in normal rats (group 1). Acute hypophysectomy (group 2) abolished the homeostatically effective increase in renal sodium excretion, and small doses of ADH failed to restore it despite decreasing the free-water excretion (group 3). Another effect of ADH was the maintaining of arterial blood pressure in the acutely hypophysectomized rats at the level found in normal animals (1 vs 3). However, this haemodynamic effect was not of decisive importance to the mechanism of the renal sodium excretion, as the rats in group 1 and 3 excreted different amounts of sodium following the saline infusion inspite of similar arterial pressures. On the other hand, the excretion of sodium was not different in rats in hypophysectomized groups (2 and 3) despite different blood pressures. There was no correlation between GFR and variable amounts of renal sodium excretion.

So, the effect of ADH in the hypophysectomized animals was similar to that found in normal saline loaded rats where also no effect of small doses of ADH on 'volume' natriuresis was found[2]. As in the present experiments, only the urine output was less pronounced in the ADH treated rats due to the increased reabsorption of free water.

It may be concluded from these experiments that the plasma level of ADH which is efficient enough to concentrate urine would not be a prerequisite for the homeostatic mechanism promoting the 'volume' natriuresis.

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Corticosterone secretion after neurogenic stress in intact and hypophysectomized rats

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Summary. The time course of blood and adrenal corticosterone elevation after immobilization stress has been studied in intact and hypophysectomized male rats. The results suggest that the adrenal gland is able to respond to neurogenic stress, increasing the synthesis and release of corticosterone, in the absence of ACTH.

It is generally accepted that the synthesis and release of corticosteroids is mediated by ACTH. Up to now, little attention has been paid to adrenal responsiveness in the absence of this hormone, and the attempts to detect changes in corticosterone levels in hypophysectomized animals subjected to neurogenic stress have proved unsuccessful[2,3]. However, the data presented here might suggest that the adrenal glands of male rats, hypophysectomized 24 h before, are able to synthesize and release corticosteroids when the animals are subjected to immobilization stress.

Male Wistar rats of similar ages were used. The transaural approach was used for hypophysectomy, and 24 h were allowed for recovery before the stress was applied. The immobilization stress was achieved by having the rats held in a prone position, with their 4 limbs fixed to a wooden board, for 5 min. The stress period commenced from the moment the animals were removed from the cage where they had been living. The survival intervals varied between 3 and 45 min. All rats that survived for more than 5 min waited in individual cages. The animals were killed by decapitation, and blood and adrenals were quickly collected. These operations were performed in a room adjacent to where the animals were caged, to minimize uncontrolled stress. Only animals whose hypophysectomy was found to be total by post-mortem inspection were used in this study.

Fluorometric measurements of corticosterone were carried out according to Demoor[4], except that readings were taken 30 min instead of 5 min after adding the sulphuric acid-ethanol reagent. The precision of the method was of 7.3%. Adrenal protein content was measured using the Lowry[5] procedure. The experiments were designed in a fully randomized way. Single (blood samples) or 2-way (left and right adrenals) analysis of variance model I were carried out after logarithmic transformation of the data, in order to avoid the heterogeneity of variances (F-max test). Since the overall analysis of variance was found significant in all the cases, and no difference was detected between left and right glands, an SNK a posteriori test was applied to study the differences among means. The results are given graphically at the bottom of figures 1 and 2. Confidence limits are given, instead of SEM, because of the logarithmic transformation required. Statistical methods were taken from Sokal[6].

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