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Excretion of Water and Sodium Loading by Isolated Perfused Dog Kidney*

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Summary. Isolated dog kidney submitted to artificial perfusion by heparinized blood is able to reject a water and sodium load. The influence of extrarenal hormonal factors is ruled out. Although the interference of lowering of haematocrit and plasma protein level and increase of plasma flow cannot be discarded, other factors related to blood dilution must play a role. Sodium and water rejection depend on a decrease of tubular reabsorption rather than on an increase in glomerular filtration.

The mechanisms insuring the rejection of water and sodium loading by the kidney are not fully elucidated. Various authors (Levinsky, Lalone and Moss [10], McDonald and DeWardener [13], Lichardus and Pierce [11], Rector, Van Giesen, Kiil and Seldin [17] admit that hormonal factors (aldosterone) do not suffice to explain the observed facts, particularly the fast variations of sodium excretion. The existence of a natriuretic factor has been postulated by De Wardener, Mills, Clapham and Hayter [4], later by Johnston an Davis [9], by Lichardus and Pearce [12].

Our previous experiments (Nizet, Cuypers, Deetjen and Kramer [16]) demonstrated that a kidney taken from a dog submitted to a salt-rich diet and artificially perfused after removal from the animal axhibits a high sodium rejection during the first hour of perfusion; the results are compatible with the presence in the blood of a natriuretic factor of relatively short life. In the same series of experiments, we observed a high sodium excretion when 500 ml of normal saline were infused to the dog 30 min before starting artificial perfusion. Moreover, high sodium output was observed after addition of 150 ml of normal saline to the blood *during* artificial perfusion. This last observation has also been made by Mills, Osbaldiston, Craig and Wise [14] and could hardly be related to the interference of a natriuretic factor.

The experiments briefly described in this paper demonstrate the response of the isolated dog kidney to a water and saline loading occur-

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ring after the removal of kidney and at the beginning of perfusion. In order to reduce the interference of previous state of blood and kidney donor animals, both kidneys of the same pair have been removed and perfused simultaneously by two identical machines, using the same perfusion blood and identical conditions of blood pressure, and only one kidney being submitted to water and sodium loading.

Material and Methods

The perfusion machine, including pump and oxygenator, has been previously described (NIZET [15], CUYPERS, NIZET and BAERTEN [3] with the conditions required to avoid vasoconstriction. Two identical machines are used simultaneously. The week preceeding the experiment, the dogs are submitted to a salt-poor diet. For each double experiment, blood is taken from the same donor different from kidney donor. Immediately before removal of kidneys and blood, the dogs receive intravenously 26 ml/kg of Pentobarbital and 25,000 I.U. of heparin. The same volume of blood (450 ml) has been used in all experiments. In order to reduce initial vasoconstriction, 25 mg Promethazine are added to the blood introduced in the perfusion equipment. In one of the machines, 150 ml normal saline are added to the blood at the beginning of the experiment, the contralateral kidney being perfused by the other machine with undiluted blood. Blood pressure is kept constant at 110 mm Hg and temperature at 37,5° C. Duration of each experiment is $2^1/_2$ hours; urine samples are collected after $^1/_2$ hour periods; blood samples are taken in the middle of each period. In each equipment, a continuous injection of a solution containing 2 g glucose, 2 g urea and 1,5 g potassium chloride per 100 ml Ringer's solution is made at a rate of 6 ml/hour.

Glomerular filtration rate is measured from creatinin clearance. A priming dose of 20 mg creatinine per 100 ml blood is added at the beginning and is followed by a continuous infusion at 6 ml/hour of a 2 p. 100 creatinine solution.

In both machines, after each 30 min period, Ringer's solution diluted to one half is added to the blood in volumes corresponding to the volume of urine excreted by the control kidney.

For all technical details, we refer to the previously cited papers.

Experimental Results

The average results of five double experiments are figured in the Table.

a) Points Common to the Control Kidney and to the Kidney Submitted to Saline Load

The general behavior of perfused kidney has been described in our previous papers [15, 16]. Renal blood flow increases progressively. Because of the lack of ADH supplementation, a water diuresis develops, with production of a hypotonic urine. In spite of replacement of urine by dilute Ringer's solution, the result is an increase in plasma osmolality and sodium content. Glomerular filtration rate is subnormal at the beginning and decreases slowly. Sodium load decreases also. There is a slight increase of sodium rejection on the control side at the end of the

Table. *Sodium and water excretion by isolated kidney with and without saline load. (Average values of 5 double experiments)*

	Control kidney (450 ml blood)				Saline load (450 ml blood + 150 ml NaCl 9 p. 1000)			
Time (minutes)	30—60	60—90	90—120	120—150	30—60	60—90	90—120	120—150
Renal blood flow (ml/g/min)	4.24	4.58	6	5,88	4.72	5.16	5.34	5.26
Haematocrit (p. 100)	52	53	52	51	41	46	51	52
Plasma Na (mEq/l)	163	167	171	166	157	171	186	187
Plasma K (mEq/l)	4.32	4.18	3.86	3.8	2.64	2.62	2.58	2.92
Plasma Cl (mEq/l)	116	119	121	125	123	127	130	131
Plasma Urea (g/l)	0.34	0.31	0.26	0.23	0.23	0.22	0.20	0.21
Plasma proteins (g/l)	71	73	71	67	49	59	71	76
Plasma osmolality (mOsm)	317	331	341	338	311	337	357	364
Urine flow (ml/100 g/min)	1.26	3.20	3.62	3.46	6.13	9.62	6.75	3.75
Urine Na (mEq/l)	47.5	28.2	28	33.8	97.4	82.8	87.8	83
Urine K (mEq/l)	38.1	15.8	12.3	13.8	11.5	6.3	6.6	8.2
Urine Cl (mEq/l)	50.6	29.7	26,9	38.3	76.8	66.4	77.8	75
Urine urea (g/l)	4.61	2.74	1.89	1.62	1.24	0.84	0.59	0.74
Urine osmolality (mOsm)	289	172	124	142	236	180	189	184
Glomerular filtration rate (ml/100 g/min)	37	56	43	32	49	54	36	26
Na Load (μEq/100 g/min)	5,891	8,769	7,464	5,438	7,613	9,164	6,618	4,921
Na rejection (p. 100 of load)	1.44	1.27	1.18	1.83	9.03	9.25	8.65	6.14
Total urine volume (ml)			115.8				287.4	
Kidney weight (g)			36.3				38	

experiment, probably because of impairment of reabsorption. However, sodium reabsorption in the control kidneys remains better than 98 per cent of sodium load.

b) Influence of Sodium Load

In the cases of total renal blood flow, glomerular filtration rate sodium load and percentage of sodium rejection, the observed differences have been analysed statistically with the help of the method of pair comparison, the experimental setup reducing the interference of individual differences between dogs. The differences are considered to be significant for $2\,P \leqslant 0.05$. Statistical analysis has been based only on the results of the second and third half-hour periods, and separately on the 4th and 5th periods for blood flow.

Blood dilution by isotonic sodium chloride induced a *decrease of haematocrit, urea, potassium and plasma protein levels.* In the course of the experiments, the elimination of excess water induces an increase of plasma protein concentration and of haematocrit up to control values. The total amount of urine excreted under saline load exceeds the volume of saline solution added. It must be pointed out that the urine is hypo tonic and that the plasma becomes hypertonic. Excess urine secretion is probably related to the sodium content at the end of perfusion.

Total renal blood flow is moderately increased ($2\,P = 0.03$) during the first 90 min. During the last hour, it decreases and falls significantly below control levels ($2\,P = 0.001$) as does plasma flow.

In spite of individual or transitory differences, *glomerular filtration* is not significantly increased ($2\,P = 0.25$) by saline dilution, blood pressure being kept constant. There is also no systematic increase of sodium load ($2\,P = 0.070$).

On the contrary, the *amount of sodium rejected*, expressed in absolute value or in per cent of sodium load, is considerably increased ($2\,P < 0{,}005$). There is a tendency to decrease at the end of perfusion, after elimination of the bulk of excess sodium and water.

Discussion

The experiments demonstrate that the isolated dog kidney, separated from the body and perfused at constant pressure by heparinized blood, is able to reject a saline load introduced at the beginning of artificial perfusion. The experimental conditions rule out the interference of extrarenal humoral factors. The experimental results are in good agreement with the findings of Mills, Osbaldiston, Craig and Wise [14] and of ourselves (Nizet, Cuypers, Deetjen and Kramer [16]). Although the mechanism of sodium rejection is not elucidated by the experiments, some conclusions are, however, permitted.

The independence of sodium rejection to glomerular filtration rate and sodium load is in good agreement with the findings of CRAWFORD and LUDEMANN [2], WIGGINS, CLAYTON, LYONS and PITTS [19], BLYTHE and WELT [1], RECTOR, VAN GIESEN, KIIL and SELDIN [17], EARLEY and FRIEDLER [6—8], WATSON [18]. The primary phenomenon in our experiments must be a decrease of tubular reabsorption, as admitted by DIRKS, CIRKSENA and BERLINER [5].

A possible role of haematocrit decrease and plasma flow increase has been postulated by EARLEY and FRIEDLER [6—8] and by MILLS and coll. [14]; it is, however, not admitted by WIGGINS, CLAYTON, LYONS and PITTS [19]. Dilution of plasma proteins also interfere with water and sodium excretion.

Although these factors are not ruled out in our experiments, they do not seem to explain entirely the induction of sodium rejection. During the last hour of perfusion, blood flow and plasma flow are lower than the control values; haematocrit and plasma protein levels have come back to control values; however, there is still a strong sodium rejection. The possible role of ionic disequilibrium should be taken into consideration, as, for example, excess sodium and chloride concentration.

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