Pflügers Archiv 301, 7—15 (1968)

Influence of Serumalbumin and Dextran on Sodium and Water Excretion by the Isolated Dog Kidney*

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Received February 23, 1968

Summary. The addition of serumalbumin or of dextran reduces sodium and water excretion by isolated kidneys submitted to a saline load. While dextran supplementation produces a considerable drop of glomerular filtration, serumalbumin supplementation induces only slight changes in filtration; a proportional increase of tubular reabsorption is observed. The difference between the response to dextran and serumalbumin may be related to differences in intrarenal blood circulation. Changes in plasma protein concentration play a role in autonomous renal response to saline loading and influence tubular sodium reabsorption. This autonomous renal response is primarily related to plasma dilution.

Key-Words: Sodium Excretion — Water Excretion — Isolated Kidney.

Schlüsselwörter: Na-Exkretion — Wasserexkretion — Isolierte Niere.

It seems reasonably well demonstrated that the immediate response of mammalian kidney to fast changes of water and sodium load cannot be related to concomitant variations of aldosterone secretion (Crabbé, Ross and Thorn [2]).

Artificial perfusion of isolated dog kidney, eliminating extra-renal somatic interferences, has demonstrated that the kidney is able to control by itself the elimination of a sodium and water load added in vitro (Mills, Osbaldiston, Craig and Wise [8]; Nizet, Cuypers, Deetjen, and Kramer [12]; Nizet [10]). Such a rejection of water and sodium excess can be observed in conditions of constant blood pressure; an increase of filtration rate may be observed, however, sodium rejection may occur without any significant increase of filtration; therefore a decrease of sodium reabsorption must play a major role (Nizet [10]). The experiments, performed at a constant blood pressure, suggest that the autonomous response of kidney to saline load is the consequence of blood changes caused by dilution. Among these changes, we have previously considered the decrease of plasma potassium concentration and we have demonstrated that acute hypokalaemia induces an increase in

* This work has been performed with the help of the "Fonds National de la Recherche Scientifique".

sodium and water excretion, this increase being the consequence of decreased tubular reabsorption (NIZET [11]). However, hypokalaemia is probably not the only one factor involved: correction of hypokalaemia does not suppress completely water and sodium rejection; moreover, decrease in plasma potassium after saline loading of the total animal is less important than after addition of normal saline to the blood in vitro.

Other consequences of blood dilution must be taken into consideration, for example, decrease of haematocrit and of plasma protein concentration. Both factors could play a role by the way of intrarenal circulatory changes caused by modified blood viscosity (THURAU [17]). Moreover, dilution might modify sodium excretion as a consequence of decrease of colloidosmotic plasma pressure. Such a change in colloidosmotic pressure might act in two ways:

a) By modifying effective filtration pressure at the glomerular level. It is well known that changes in sodium excretion may be induced by changes in glomerular filtration (SELKURT and coll. [15]; THOMPSON and PITTS [16]).

b) By modifying the rate of passive transtubular sodium reabsorption due to transtubular oncotic gradient. Such transtubular passive reabsorption has been demonstrated by VOGEL et al. [21,22] in amphibians and by VEREERSTRAETEN and TOUSSAINT [20] in birds. The role of passive reabsorption has been discussed by VANDER et al. [18].

EARLEY [5] observed a decrease in sodium excretion after renal arterial infusion of albumin but was unable to separate the two factors: filtration and reabsorption. In a recent paper, VEREERSTRAETEN, DE MYTTENAERE and LAMBERT [19] have demonstrated that protein infusion into dog renal artery induces a decrease in sodium excretion independent from filtration changes and therefore dependent on increased reabsorption, whatever the intimate mechanism of this increase might be.

The purpose of the experiments described in the present paper is to demonstrate the influence of changes in protein concentration and colloidosmotic plasma pressure on the rejection of saline load by the isolated dog kidney under conditions of constant blood pressure.

Material and Methods

Dog kidneys are perfused by an artificial pump-oxygenator system with heparinized blood at a temperature of 37.5 °C. The equipment has been previously described (CUYPERS, NIZET and BAERTEN [3]). In order to reduce the influence of individual animal differences, both kidneys of the same pair are perfused simultaneously by two identical machines, under identical conditions and only one factor being modified on one side. For each double experiment blood is furnished by one donor different from kidney donor. Both blood and kidney donors are submitted to a low sodium diet during eight days prior to the experiment. Immediately before blood and kidneys removal, the dogs receive intravenously 26 mg/kg Pentobarbital

and 25,000 I.U. Heparin. In order to reduce initial vasoconstriction 25 mg Promethazine (Phenergan Specia) are added to the blood introduced in each machine (cf. NIZET, CUYPERS, DEETJEN, and KRAMER [12]).

Blood perfusion pressure is kept constant at 110 mm Hg. A constant volume of blood (450 ml) is used in all experiments. A solution containing 2 g glucose, 1 g urea and 2 g potassium chloride per 100 ml Ringer's solution is continuously injected at a rate of 6 ml/hour. Glomerular filtration is evaluated from creatinine clearance. A priming dose of creatinine (20 mg/100 ml of blood) is introduced at the beginning of perfusion and followed by continuous injection of 6 ml/hour of a 2 per cent solution. The duration of each double experiment is 2 hours. Urine samples are collected during 30 min periods; blood samples are taken in the middle of each period.

In each double experiment we have added on both sides at the beginning of perfusion 150 ml of 0.9 per cent NaCl solution. In order to avoid hypokalaemia, 56 mg potassium chloride are added on both sides. On one side, the normal saline has been supplemented either by human serumalbumin or by dextran.

The urine excreted is periodically replaced by half diluted Ringer's solution in amounts equal to the amount of urine emitted on the side corresponding to serumalbumin or dextran supplementation.

In the absence of antidiuretic hormone, a state of water diuresis develops with emission of hypotonic urine: in spite of replacement by half diluted Ringer's solution, plasma osmolality increases steadily.

For total renal blood flow, glomerular filtration rate, sodium load and percentage of sodium rejection, the observed differences have been analyzed 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 $2p \leqslant 0.05$. Statistical analysis has been based only on the results of the second and third half-hour periods.

1. Dextran Supplementation

The results of 5 double experiments are given in Table 1. The amount of dextran added (Fluka, average molecular weight 80,000) is 8.4 g for 150 ml normal saline.

In the presence of dextran, total renal blood flow is not significantly reduced ($2p = 0.6$). Haematocrit is reduced because of increased sedimentation of red blood cells at the bottom of the oxygenator. Urine volume is strongly reduced (75 ml against 253 ml); replacement by 75 ml half diluted Ringer only on both sides does not avoid increase of plasma sodium and osmolality, which is very considerable on control side. In spite of this, there is a significant decrease in filtration rate ($2p = 0.005$), sodium load ($2p = 0.005$) and sodium rejection ($2p < 0.005$) in the presence of dextran.

2. Serumalbumin Supplementation

The average results of 4 double experiments are given in Table 2.

The amount of human serumalbumin added (Mérieux, Lyon, France) is 6 g per 150 ml normal saline. Serumalbumin is previously dialyzed during 24 hours against normal saline in order to remove stabilizer (Sodium caprylate).

Table 1. *Influence of dextran on the excretion of saline load. (Average values of 5 double experiments)*

	450 ml Blood + 150 ml normal saline			450 ml Blood + 150 ml normal saline + 8.4 g dextran		
Time (min)	30—60	60—90	90—120	30—60	60—90	90—120
Rénal blood flow (ml/g/min)	6.03	6.65	6.46	6.03	6.86	7.52
Haematocrit (p. 100)	38.8	42.8	48.4	34.4	34.2	34.4
Plasma Na (mEq/l)	157	181	201	150	155	163
Plasma K(mEq/l)	4	4.2	4.1	4.8	5.2	5.7
Plasma Cl (mEq/l)	131	143	160	118	132	138
Plasma urea (g/l)	0.31	0.26	0.24	0.38	0.38	0.36
Plasma osmolality (mOsm)	313	343	390	308	318	337
Urine flow (ml/100 g/min)	5.90	8.52	5.40	0.72	1.79	2.18
Urine Na (mEq/l)	89.2	67.4	79.9	51.7	17.7	13.1
Urine K (mEq/l)	20.1	12.8	15.7	49.6	16.3	12.1
Urine Cl (mEq/l)	98	81	109	103	61	60
Urine urea (g/l)	2.13	1	0.76	5.15	2.79	2.14
Urine osmolality (mOsm)	243	170	193	374	208	144
Glomerular filtration (ml/100 g/min)	56.1	56.7	36.9	32.2	33	30.4
Na Load (μEq/100 g/min)	8,854	10,310	7,130	4,861	5,093	5,375
Na rejection (p. 100 of load)	5.94	5.59	6.05	0.77	0.62	0.54
Total urine volume (ml)		253			75	
Kidney weight(g)		37.4			39.3	

The volume of urine emitted within 2 hours is 124 ml with albumin supplementation and 275 ml without supplementation. Quantitative replacement of urine on the serumalbumin side has kept haematocrit practically constant; a slight decrease may be explained by erythrocyte plasmolysis due to increased plasma osmolality. On the control side,

Table 2. *Influence of serumalbumin on the excretion of saline load. (Average values of 4 double experiments)*

	450 ml blood + 150 ml normal saline			450 ml blood + 150 ml normal saline + 6 g serumalbumin		
Time (min)	30—60	60—90	90—120	30—60	60—90	90—120
Renal blood flow (ml/g/min)	6.3	6.8	7.4	8.1	8.8	8.9
Haematocrit (p. 100)	42.5	45.5	51.5	41.2	40.8	40.2
Plasma Na (mEq/l)	147	157	171	149	153	161
Plasma K (mEq/l)	3.7	3.4	3.5	3.8	3.9	4
Plasma Cl (mEq/l)	125	134	148	120	124	134
Plasma urea (g/l)	0.27	0.19	0.18	0.24	0.22	0.21
Plasma osmolality (mOsm)	304	327	361	302	312	321
Plasma proteins (g/l)	50.6	58.2	70.2	67	68	65.9
Urine flow (ml/100 g/min)	4.82	9.01	8.03	1.68	3.3	3.9
Urine Na (mEq/l)	93.4	64	68.8	90.5	36.4	14.6
Urine K (mEq/l)	20.2	8.1	6.7	51	28.4	8.1
Urine Cl (mEq/l)	85.9	60.4	65.9	97.6	44.9	17.9
Urine urea (g/l)	1.7	0.67	0.51	4.32	4.34	2.38
Urine osmolality (mOsm)	249	152	150	390	247	108
Glomerular filtration (ml/100 g/min)	58.3	62.4	48.3	54.1	55	46.3
Na load (μEq/100 g/min)	8,565	9,920	8,553	8,067	8,512	7,793
Na rejection (p. 100 of load)	5.25	5.81	6.45	1.88	1.41	0.73
Total urine volume (ml)	275			124		
Kidney weight (g)	39.6			39		

excess elimination of 151 ml liquid resulted in a considerable increase of haematocrit, plasma sodium, chloride and osmolality.

Serumalbumin addition resulted in a significant increase in total renal blood flow ($2\,p = 0.001$); we have no satisfactory explanation for

this increase, but it is known that addition of foreign macromolecules induces various changes in kidney blood circulation (O'Connor [13]). Glomerular filtration rate is moderately decreased by serumalbumin for each corresponding period; however, the difference is at the limit of significance ($2\,p = 0.08$). Sodium load is also lower but differences are not statistically significant ($2\,p = 0.75$). Sodium rejection is considerably reduced by serumalbumin supplementation ($2\,p < 0.005$); this finding implies an increase in fractional sodium reabsorption. Differences in plasma sodium, potassium, chloride and urea during the second and third $^1/_2$ hour periods are of little significance.

Discussion of Results

In the present experiments, performed at a constant arterial pressure, it is conceivable that the addition of foreign macromolecules (human serumalbumin or dextran) could reduce sodium excretion by reduction of glomerular filtration, increased tubular reabsorption or both. The modifications could be the consequence of increased colloidosmotic pressure (whatever the mechanism may be) or of haemodynamic intrarenal changes.

It can be calculated that the addition of dextran produces an increase of about 5 mm Hg of colloidosmotic pressure in the plasma. This increase could explain the drop of filtration on the basis of a decrease in effective filtration pressure; lowered filtration gives a possible explanation for decreased sodium rejection. However, many other intrarenal changes may be the consequence of the introduction of abnormal macromolecules in the blood. The only possible conclusion of the present experiments is that the pharmacological effect of dextran supplementation after loading of isolated kidney with normal saline is a decrease in sodium and water excretion together with a decrease of glomerular filtration. It is not possible to know whether these changes are entirely related to changes of oncotic pressure or to other consequences of the presence of dextran. It should be pointed out that decrease of saline excretion by dextran is observed in a preparation working at constant blood pressure and eliminating the exchanges between vascular and extravascular spaces. These exchanges occurring in the whole body may result in increased plasma volume, sodium displacement from extravascular spaces and, finally diuretic and saluretic effect.

The experiments involving serumalbumin supplementation are of greater physiological significance. The increment of plasma oncotic pressure due to serumalbumin, calculated according to Key's formula, is of 6 mm Hg on the average during the first period of urine collection. Despite this considerable increase of oncotic pressure (of about 37 p. 100) there is only a moderate change of filtration rate. Blood flow (and plasma

flow) are considerably higher than on the control side; it is possible that a change in pre- and postglomerular blood pressure gradients has increased hydrostatic component of effective filtration pressure and compensated for increased oncotic pressure. In strong contrast with the moderate decrease of filtration and sodium load, the percentage of sodium rejection is considerably lowered.

This finding indicates an increase of proportional reabsorption. It is particularly suggestive to compare the datas collected during the second collection period (60—90 min) on the serumalbumin side and during the first collection period (30—60 min) on control side. In the presence of serumalbumin, blood flow is higher, plasma sodium and osmolality slightly higher, haematocrit slightly lower; in spite of this and of identical sodium load there is an increase, not only of percentage of sodium reabsorbed, but also of absolute reabsorption.

Increased reabsorption may be purely passive and due to the oncotic gradient but changes in active process, possibly mediated by vascular changes, are conceivable (VEREERSTRAETEN, DE MYTTENAERE, and LAMBERT [19]).

It may be concluded that the reduction of plasma protein concentration is one of the blood changes responsible for sodium and water rejection by the kidney after saline loading; this rejection is at least partly due to decreased sodium reabsorption. The fall in plasma protein concentration is likely to act through decreased oncotic pressure, other mechanisms being not excluded.

ROSENFELD, KRAUS, and MCCULLEN [14] working in conditions of constant renal blood flow observed that a 25 p. 100 increase in plasma protein concentration induces a decrease of 48 p. 100 of filtration rate and of 72 p. 100 of sodium excretion. Many conflicting results are found in the literature. For CIZEK and ZUKER [1] a fall in plasma protein concentration is not associated with a rise in sodium excretion if there is no decrease in blood volume. For MILLS, DE WARDENER, HAYTER, and CLAPHAM [9] and for LEVINSKY, LALONE and MOSS [6] the infusion to the dog of albumin together with normal saline increases sodium excretion similarly to saline alone. According to MACDONALD, and DE WARDENER [7], the increase in sodium excretion which occurs during an infusion of saline is not related to creatinine clearance, renal blood flow, filtered sodium, potassium excretion, PAH extraction or fall of packed cell volume; these authors find a positive relation with blood pressure and a significant inverse correlation between the rise in sodium excretion and the fall in plasma protein concentration. However, according to DE WARDENER, MILLS, CLAPHAM, and HAYTER [4] blood pressure increase is not the main factor.

Many of the differences can be explained by the interference of extrarenal influences in experiments involving the whole animal. It is obvious that the injection of serumalbumin into the blood stream of the whole body induces various changes (extravascular sodium displacement, increase in plasma volume and blood pressure) that compete with the immediate renal effect.

The experiments, performed with totally isolated kidneys demonstrate that this organ, working in experimental conditions which exclude blood pressure and volume changes and extrarenal humoral factors is able to control the elimination of a saline load by autonomous mechanisms. Ionic disequilibrium, as for example plasma potassium dilution, and plasma protein dilution promote sodium rejection. Changes in plasma flow and in haematocrit are also likely to play a role. However, one of these parameters alone should not suffice, otherwise one should expect to observe sodium losses in anaemia or hypoproteinemia.

There is an increasing probability that the fast response of kidney to saline loading is an autonomous response to cumulative factors bound to blood dilution more than to volume or pressure changes.

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