Natriuretic hormone—its possible role in fluid and electrolyte disturbances in chronic liver disease

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Summary

Besides intrarenal physical factors and aldosterone, a natriuretic hormone has been postulated to modulate renal tubular sodium resorption in order to maintain body fluid homeostasis. To investigate the possible role of a natriuretic activity in sodium retention of chronic liver disease, the effects of plasma and plasma fraction IV from patients with cirrhosis of the liver and ascites on sodium transport of the isolated frog skin and on renal sodium excretion in the rat were compared to the antinatriuretic and natriuretic effects of plasma from healthy subjects. While plasma from healthy individuals obtained following acute expansion of the extracellular fluid volume (ECV) significantly inhibited potential difference (PD) by $-43.8 \pm 5.5\%$ and short circuit current (SCC) by $-41.3 \pm 1.7\%$ when applied to the inner skin surface, control plasma and plasma from patients with liver cirrhosis and ascites affected PD by $-3.8 \pm 4.7\%$ and $-5.2 \pm 3.7\%$ and SCC by $-7.3 \pm 4.6\%$ and $-11.7 \pm 2.5\%$ respectively.

Similar effects on PD and SCC were observed with plasma fractions IV.

In contrast to fraction IV from ECV-expanded individuals, which caused marked diuresis and natriuresis when injected in the rat, fraction IV of plasma from cirrhotic patients failed to affect urinary flow rate, free-water clearance or renal sodium excretion. The results suggest that at least some patients with cirrhosis of the liver and sodium retention may lack an adequate humoral natriuretic activity sufficiently to promote renal sodium excretion.

Introduction

In chronic liver disease, abnormal retention of salt and water with limited natriuretic response to sodium load (Goodyer *et al.*, 1950) is ascribed to impaired renal function, eventually leading to progressive oliguric renal failure. Although a recent report suggests that enhanced distal tubular sodium resorption is primarily responsible for fluid retention in some

patients with liver disease (Chaimovitz *et al.*, 1972), avid proximal tubular sodium resorption has been incriminated on the basis of previous observations on renal sodium and free-water clearance in these patients (Baldus *et al.*, 1964a; Summerskill, 1966; Vesin, 1972).

While the roles of aldosterone (Wolff *et al.*, 1966) and of intrarenal physical factors (Schrier and de Wardener, 1971) in salt and water homeostasis are well established and, by enhancing distal and proximal tubular sodium resorption, appear to be predominantly responsible for avid salt retention in advanced chronic liver disease, antidiuretic hormone does not seem to play an important role with respect to impaired water balance observed in hepatic failure (Vaamonde *et al.*, 1971). Additional factors, however, must be evoked to explain positive sodium balance already noted during the early course of chronic liver disease (stage I according to Vesin, 1972) when renal blood flow, glomerular filtration rate and filtration fraction (Vesin, 1972), peritubular hydrostatic and oncotic pressure may be normal.

In addition, the enhanced proximal tubular sodium resorption in chronic liver disease, may depend on a yet unidentified humoral factor. To investigate this hypothesis in the present study, the effects of plasma and plasma fraction IV from patients with cirrhosis of the liver and ascites on sodium transport of the isolated frog skin and on renal sodium excretion in the rat were studied. They were compared to the effects of plasma from healthy individuals and of plasma fraction IV previously shown to contain the antinatriuretic and natriuretic activity present in plasma from ECV expanded subjects.

Materials and methods

Six healthy human subjects were infused intravenously with 1 litre of isotonic Ringer solution within 30 min followed by 2 litres of Ringer solution administered during the next 2 hr. Fifty millilitres of venous blood were drawn before and at the end of each study. Plasma samples are subsequently designated as control and natriuretic plasma respectively.

From seven patients with cirrhosis of the liver, six

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TABLE 1. Body weight before and after diuretic therapy and blood chemistry on admission of patients with liver cirrhosis

Patient	Age (years)	Body weight		Serum						
		Before therapy (kg)	After therapy (kg)	Na (mEq/l)	K (mEq/l)	BUN (mg %)	Albumin (g %)	Bilirubin (mg %)	SGOT (mu/ml)	Prothrombin (%)
Ru.	49	68.0	56.4	140	4.7	24	2.6	3.0	34	46
Wa.	62	77.4	66.9	137	4.4	28	1.8	3.6	140	44
Ko.	61	57.5	47.0	140	2.6	23	2.8	8.2	27	39
Sch.	63	96.9	87.2	139	5.2	23	3.8	0.7	34	86
Kl.	60	65.0	50.6	137	4.4	36	2.2	1.0	24	74
Ba.	50	53.1	47.3	124	3.2	25	2.6	3.7	58	52
Bu.	52	82.5	79.5	142	3.8	22	3.0	1.1	53	60

with ascites and peripheral oedema and one (Bu.) without clinical manifestation of fluid retention, blood was drawn before initiation of therapy. Fluid retention was estimated from weight loss during diuretic therapy at the time when body weight had stabilized in the absence of demonstrable ascites. The diagnosis of cirrhosis of the liver was based on previous history, physical examination, blood chemistry (Table 1) and histological examination.

Blood samples were immediately placed in ice and centrifuged at 4°C. Plasma was frozen stored at -18°C. Plasma samples (10 ml) were fractionated by column chromatography using Sephadex G-25® as reported by Kramer, Gospodinov and Krück (1974). Single fractions pooled to fraction IV (Fig. 1) were lyophilized and the resulting powder was stored at -18°C. The effects of plasma and plasma fraction IV on PD and SCC of the isolated abdominal skin

of the frog, *Rana temporaria*, were investigated as described by Kramer *et al.* (1974).

To study natriuretic activity of plasma fraction IV, female Sprague Dawley rats weighing approximately 250 g with previously free access to food and water were employed. Under light ether anaesthesia, a polyethylene catheter (PE 50) was inserted into the jugular vein and an additional catheter (PE 90) was placed transabdominally into the urinary bladder. The animals were then placed in single restraining cages. After i.v. injection of 2 ml of isotonic saline to replace volume loss during surgery, an infusion of 2.5% glucose solution was started to maintain body weight constant throughout the experiment. One hour after surgery was completed, when urinary flow rate and sodium excretion had stabilized, 0.5 ml of lyophilized and ten-fold concentrated plasma fractions IV reconstituted with distilled water (pH 7.2) or 0.5 ml of 2.5% glucose solution were injected i.v. followed by four to six 15-min urine collection periods.

Concentrations of sodium and potassium were determined by flame photometry. A Beckman pH micro-electrode was used for pH determination and an advanced osmometer was used for osmolality determination. Glomerular filtration rate was measured as described by Kramer and Gonick (1974). The results were analysed statistically using double tail Student's *t*-test. Data are presented as mean $\pm$ s.e. mean.

Results

The effects of plasma from six healthy human subjects obtained before and after acute expansion of the ECV on PD and SCC of the isolated frog skin are summarized in Fig. 2. The Ringer solution bathing the inside surface of the skin was replaced by 10 ml of post-expansion plasma, then an immediate fall in PD and SCC was noted, while control plasma had no significant effect. After 60 min, mean changes in PD and SCC by control plasma were $-3.8 \pm 4.7\%$ and $-7.3 \pm 4.6\%$, respectively, whereas natriuretic plasma depressed PD by $-43.8 \pm 5.5\%$ and SCC by

FIG. 1. Fractionation patterns of human plasma following gel filtration through Sephadex G-25. Presented are UV₂₈₀-absorption, ninhydrin reaction and calcium concentrations of single fractions subsequently pooled to fractions I to V.

TABLE 2. Effects of plasma from patients with liver cirrhosis and ascites on potential difference (PD) and short-circuit current (SCC) of isolated frog skin as compared to the effects of plasma from control subjects before and after ECV expansion

Patient	Controls				Patients with cirrhosis of the liver and ascites		
	Pre-expansion		Post-expansion				
	PD	SCC	PD	SCC	Patient	PD	SCC
Na.	+16	+4	-44	-38	Ru.	-14	-17
Go.	-2	0	-46	-48	Wa.	-14	-16
Po.	-14	-25	-34	-36	Ko.	-7	0
Go.	-8	+1	-29	-43	Schw.	+10	-12
Si.	0	-16	-68	-40	Kl.	0	-13
Sp.	-5	-8	-42	-43	Ba.	-6	-12
Mean	-3.8	-7.3	-43.8*	-41.3*		-5.2	-11.7
s.e.	4.7	4.6	5.5	1.7		3.7	2.5

* $P < 0.001$.

FIG. 2. Mean decrease in PD and SCC of the isolated frog skin following addition of plasma from healthy subjects obtained before and after acute ECV-expansion. The inhibition of sodium transport is reversible when plasma is replaced by fresh Ringer solution. $\bigcirc$ - - - $\bigcirc$, Pre-expansion; $\bullet$ - - - $\bullet$, post-expansion. */ s.e.

$-41.3 \pm 1.7\%$ ($P < 0.001$). This inhibition of sodium transport was completely reversible when plasma was replaced by fresh Ringer solution. To study reproducibility and stability of the antinatri-feric activity during storage, identical plasma samples were assayed after 6 weeks and 6 months of storage at -18°C . As shown in Fig. 3, a small time-de-pendent decrease of the effect of natriuretic plasma on

FIG. 3. Reproducibility and stability of antinatriuretic plasma activity after storage at -18°C for various intervals of time. $\bullet$ - - - $\bullet$, Within 1 week; $\triangle$ - - - $\triangle$, after 6 weeks; $\times$ - - - $\times$, after 6 months.

SCC was noted, while a more pronounced and pro-gressive loss of inhibition of PD resulted. As compared to the effects of natriuretic plasma, plasma from patients with liver cirrhosis and ascites had no significant antinatriuretic effect (Table 2). Sixty minutes after replacement of Ringer solution by plasma from patients with liver cirrhosis, mean changes of PD of $-5.2 \pm 3.7\%$ and of SCC of $-11.7 \pm 2.5\%$ were not significantly different from

FIG. 4. Mean changes in PD and SCC of isolated frog skin induced by fraction IV of plasma from healthy subjects before and after ECV-expansion and from patients with liver cirrhosis and ascites when added to the Ringer solution bathing the inner surface. ●—●, controls; ---●, ECV-expansion;●, cirrhotics. */ s.e., $n = 6$ (number of subjects).

the effects of control plasma. In contrast, the only patient (Bu.) without clinical evidence of ascites or oedema revealed an adequate antinatriuretic plasma activity with inhibition of PD by 44%, and of SCC by 47%.

Similar results were obtained when lyophilized plasma fractions IV were added to the Ringer solution bathing the inner surface of the skin (Fig. 4). Again fraction IV derived from natriuretic plasma markedly inhibited PD and SCC, while control fraction IV had no significant effect. No inhibitory activity on sodium transport of the isolated skin was observed when fraction IV of plasma from patients with cirrhosis and ascites was added to Ringer solution (Fig. 4).

When 0.5 ml of reconstituted plasma fractions IV from cirrhotic patients with ascites, corresponding to 5.0 ml of original plasma, were injected i.v. into Sprague Dawley rats, no significant increase in sodium excretion was observed when compared to two preceding urine collections taken during continuous infusion of 2.5% glucose solution to maintain a constant body weight. With one exception, urinary flow rate and potassium excretion slightly decreased following injection of fraction IV (Fig. 5). Figure 6 summarizes the changes in urinary flow rate, free-water clearance, and sodium and potassium excretion during the first 15-min collection period after injection of 0.5 ml of plasma fractions IV—corresponding to 5.0 ml of original plasma activity—from healthy subjects before and after acute ECV expansion and from cirrhotic patients with ascites. While control fraction IV did not significantly alter urinary water and electrolyte excretion, fraction IV of natriuretic plasma markedly enhanced urinary flow rate,

free-water clearance, and sodium excretion. Fraction IV of plasma from cirrhotic patients revealed a similar lack of natriuretic activity as did control fraction IV.

Discussion

Mounting evidence suggests that the liver participates directly or indirectly in the humoral regulation of body homeostasis which, for example, includes metabolism of histamine, glucocorticoids, and catecholamines. With special regard to salt and water homeostasis, this regulatory function comprises metabolism of renin (Heacox, Harvey and Vander, 1967; Wernze *et al.*, 1972), aldosterone (Wolff *et al.*, 1966), and ADH (Lauson, 1967), thereby influencing renal salt and water excretion. The liver is also involved in the production or stimulation of diuretic activity (Haberich *et al.*, 1965; Milies, 1960), which suggests its osmoregulatory role in body fluid balance (Wollheim, 1961). Furthermore, it has been suggested that the liver synthesizes a vasodepressor which regulates cardiorenal function (Baez, Mazur and Shorr, 1950); it may also play a role in the maintenance of normal renal viability (Mondon, Burton and Ishida, 1969). Thus, ample evidence can be presented for an intimate metabolic and functional interrelationship between these two organs.

Since consistent histopathological alterations of the kidney in hepatic failure have not been observed (Papper, 1973; Salomon *et al.*, 1965), kidneys grafted from patients with chronic liver disease regain normal function (Koppel *et al.*, 1969), and spontaneous recovery of renal function in liver cirrhosis has been repeatedly reported (Goldstein and Boyle,

FIG. 5. Representative time sequence of changes in urinary flow rate, and renal sodium and potassium excretion in rats following i.v. injection of 0.5 ml of reconstituted plasma fraction IV from patients with liver cirrhosis and ascites.

FIG. 6. Mean changes in urinary flow rate, free-water clearance, and sodium and potassium excretion in the rat 15 min after i.v. injection of fraction IV of control and natriuretic plasma from healthy subjects and of plasma from patients with liver cirrhosis and ascites. □, Controls ($n = 7$); ▨, cirrhotics ($n = 6$); ▩, acute ECV-expansion ($n = 7$). *% change from baseline, † s.e.; # $P < 0.05$.

1965; Pecikyan, Kanzaki and Berger, 1967), renal impairment seems a reversible functional abnormality.

Primarily, haemodynamic circulatory alterations have been evoked as responsible factors of enhanced sodium resorption in liver disease, i.e. decreased renal blood flow (Epstein *et al.*, 1970) resulting from reduced or shunted 'effective' intravascular volume (Tristiani and Cohn, 1967; Papper and Vaamonde,

1968) in the presence of elevated total plasma volume (Lieberman, Ito and Reynolds, 1969; Lieberman and Reynolds, 1967; McCloy *et al.*, 1967) and high cardiac output (Papper, 1973). In addition, increased renal vascular resistance (Baldus *et al.*, 1964b), probably at the afferent arteriolar level, and increased peritubular oncotic pressure resulting from increased filtration fraction (Summerskill, 1966;

Traverso *et al.*, 1966) have been observed, although the latter may be counteracted by hypoalbuminaemia associated with chronic hepatic failure (Brenner & Troy, 1971). Finally, the role of abnormal distribution of renal blood flow with preferential juxta-medullary and medullary perfusion in patients with liver cirrhosis has recently been emphasized (Schröder *et al.*, 1967).

Since the early 1960s, several groups of investigators have presented evidence favouring the existence of a humoral natriuretic factor (de Wardener *et al.*, 1961) which, in addition to known determinants such as glomerular filtration and circulating aldosterone activity (Smith, 1957), can inhibit tubular sodium resorption in the rat (Brown, Koutsaimanis and de Wardener, 1972; Kramer *et al.*, 1974; Sealey *et al.*, 1971), PAH (Bricker *et al.*, 1968) and sodium transport by slices of rabbit kidney (Robson *et al.*, 1969) and sodium transport by isolated biological membranes (Buckalew, Martinez and Green, 1970; Clarkson and de Wardener, 1972; Kramer and Gonick, 1974; Kramer *et al.*, 1974).

In the present study, plasma and plasma fraction IV obtained from healthy subjects following acute expansion of the extracellular fluid compartment significantly inhibited PD and SCC of the isolated skin of *Rana temporaria*. This inhibition of sodium transport was completely reversible when natriuretic plasma was replaced by Ringer solution. In contrast, plasma and plasma fraction IV from most patients with liver cirrhosis and ascites did not significantly alter PD or SCC indicating a similar lack of anti-natriuretic activity as was noted with plasma from healthy subjects before ECV expansion and, as previously reported, with plasma from patients with the nephrotic syndrome and excessive extracellular fluid retention due to oedema formation (Kramer *et al.*, 1974). In the rat bioassay no significant natriuresis was observed following i.v. administration of plasma fraction IV from cirrhotic patients. Mean changes in urinary flow rate, free-water clearance and sodium excretion were similar to those noted after injection of control fraction IV, while fraction IV derived from natriuretic plasma markedly increased urinary output, free-water clearance and sodium excretion in the absence of changes in GFR (Kramer *et al.*, 1974). Thus, it seems that at least some patients with cirrhosis of the liver and ECV expansion have no anti-natriuretic and natriuretic plasma activity or, if they do, it is inordinately low, when compared to the humoral activity demonstrated in healthy subjects following expansion of the extracellular fluid volume.

Studies from this laboratory have recently shown that this natriuretic activity can be ascribed to one plasma fraction IV obtained by gelfiltration with Sephadex G-25 (Kramer *et al.*, 1974).

On the basis of gelfiltration studies and passage

through Ultraflo® membranes the active compound has an estimated molecular weight of 1000 (Kramer, Gonick and Krück, 1972; Kramer *et al.*, 1969). It is inactivated by incubation with chymotrypsin while resistant to trichloroacetic acid. This fraction was also shown significantly to inhibit renal Na-K-ATPase *in vitro* (Kramer, 1972; Kramer *et al.*, 1969) and to possess saluretic activity in the rat bioassay (Kramer *et al.*, 1974), where increased excretion of sodium and the increase in free-water clearance suggest an inhibitory action on proximal tubular sodium re-sorption.

With respect to the possible role of such a natriuretic activity in liver disease, the key question arises of where the hypothetical hormone originates. Although so far only conflicting results have been reported, essentially two organs besides the kidney itself (Mills, Wilson and de Bono, 1971) have been implicated as possible sources: the hypothalamic area or posterior pituitary gland, and the liver. These implications are based on indirect evidence from infusion studies, on bioassay studies of natriuretic activity derived from blood leaving various organs, and on organ ablation. Thus, Cirkensa, Dirks and Berliner (1966) concluded from their experiments in the caval dog that a humoral natriuretic substance might be released from the liver. Daly, Roe and Horrocks (1967), infusing 5% saline into the femoral and portal vein, observed a significantly greater natriuretic response from the portal vein. This was confirmed by Strandhoy and Williamson (1970) when infusion of saline into the portal vein of group I and into the right atrium of group II animals gave a similar natriuretic effect but with a higher baseline sodium excretion in group II. The authors conclude from their data that the liver may be involved in the regulation of renal sodium excretion. More direct evidence on the basis of bioassay data was presented by Stahl *et al.* (1967), who found a natriuretic and diuretic activity first appearing in hepatic venous blood of volume-expanded dogs and humans. Investigating the natriuretic effects of various organ extracts in the rat, Sealey and Laragh (1971) also observed maximum natriuretic activity being present in tissue extracts of the liver.

Therefore, if a natriuretic humoral factor would be generated or released by the liver in response to changes in extracellular fluid volume, failure of renal sodium excretion in liver disease could then easily be related to the metabolic and functional impairment of this organ.

Alternatively, the demonstration of natriuretic activity isolated from blood leaving the brain suggests that this organ is the site of origin. Thus, Cort *et al.* (1968) and Buckalew *et al.* (1970) observed maximum natriuretic activity in jugular venous blood and, recently, Gitelman and Blythe (1972),

Quamme, Dirks and Friesen (1973), and Robinson *et al.* (1973) demonstrated natriuretic activity in tissue extracts of hypothalamus and posterior pituitary gland. On the basis of organ ablation, however, Levinsky (1966) concluded that a natriuretic factor is secreted neither from the brain nor the liver.

If, therefore, the liver itself would not release or stimulate the release of a natriuretic factor, how then can the lack of natriuretic response in liver disease (Goodyer *et al.*, 1950), despite an excessive retention of salt and water, be explained if we accept the hypothesis that a humoral factor participates in proximal tubular resorption of sodium? At this point it seems appropriate to consider an analogous experimental model associated with the formation of ascites. Following constriction of the thoracic inferior vena cava in the dog, which causes marked ascites, the natriuresis usually associated with ECV expansion is almost completely blunted (Cirkensa *et al.*, 1966; Pearce and Sonnenberg, 1965). This is due to avid sodium resorption in the proximal tubule (Cirkensa *et al.*, 1966) as well as to enhanced sodium absorption in the diluting segment of Henle's loop and the distal site of Na-K exchange (Kaloyanides *et al.*, 1969). Under comparable experimental conditions, Kaloyanides and Azer (1972) and Buckalew and Lancaster (1971) also failed to demonstrate a humoral activity previously observed in ECV expansion (Buckalew *et al.*, 1970). A similar effect was demonstrated during constriction of the superior vena cava (Schrier *et al.*, 1971) but was not achieved following abdominal cava constriction although leading to an equal rise in renal venous pressure (Cirkensa *et al.*, 1966).

Finally, renal nerves were shown to constitute an important efferent pathway through which acute caval constriction stimulates renal sodium retention (Azer, Gannon and Kaloyanides, 1972). In addition, the natriuretic response to ECV expansion is blunted by high spinal cord section (Pearce and Sonnenberg, 1965) and chronic cardiac denervation (Gilmore and Daggett, 1966; Knox, Davis and Berliner, 1967), while selective preservation of sympathetic efferent pathways or vagotomy do not impair the natriuretic response. This response may therefore be assigned in part to adrenergic efferent pathways of the autonomous nervous system (McDonald *et al.*, 1970).

Regional circulatory alterations in chronic liver disease might well be comparable to those seen in the caval dog. Improvement of renal function in cirrhosis [either spontaneous (Goldstein and Boyle, 1965; Pecikyan *et al.*, 1967) or following cross circulation (Burnell *et al.*, 1967)] as well as functional improvement [following side-to-side rather than end-to-side portocaval shunt (Orloff, 1966; Schröder, Numann and Chamberlain, 1970; Wolfman, Zuidema and Child, 1966)] with restored renal sodium excretion

and disappearance of ascites, might be considered in this context as a result of restored intrathoracic haemodynamics. Decreased perfusion of the liver due to portal hypertension may cause haemodynamic alterations with stimulation or suppression of thoracic venous or intracardiac volume-receptors, leading to depression of a humoral natriuretic activity. In analogy to this proposed interrelationship between formation of ascites in liver cirrhosis and renal sodium retention, Cirkensa *et al.* (1968) concluded, from their experiments in the caval dog, that the differing effects of thoracic and abdominal cava obstruction on proximal tubular sodium resorption suggest that haemodynamic alterations of caval obstruction on hepatic venous or central venous volume may be involved in stimulating or suppressing the production of a humoral factor. A more precise knowledge of the role of such a humoral mechanism, however, must await further investigation.

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