PART IX

NEPHROLOGY 1

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PATHOGENIC FACTOR IN THE SERUM OF PATIENTS WITH ACUTE RENAL FAILURE?

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Summary

Recently substances other than ions were detected in the serum of patients with acute renal failure, which suppressed single nephron GFR by stimulating the glomerular feedback mechanism at the macula densa site. Since an important role in the development of certain forms of acute renal failure has been attributed to these substances, the present experiments were performed using deionised serum samples from patients with post-operative acute renal failure. Sera which produced feedback stimulation were selected in order to characterise the constituents responsible as well as to determine their mode of action. Feedback operation was measured in cortical nephrons of the rat kidney by continuously monitoring the early proximal stop flow pressure (SFP) during perfusion of Henle's loop with deionised serum preparations. Feedback stimulation was found to be operative at loop perfusion rates between zero and 10 nl/min and increased only a little more when perfusion rates were increased further. This feedback inducing effect could not be inhibited by intratubularly applied furosemide (1–3 mM), but was effectively reversed by intravenous infusion of verapamil (0.04 mg/min/kg b.w.) or by simultaneous intratubular application of theophylline (1–5 mM). Equilibration with acrylic hydrogel coated charcoal (Haemocol ®) for 1 hour reduced the feedback-inducing effect considerably. Our findings confirm the existence of serum constituents in certain patients with p.o. ARF. Further evidence is needed to demonstrate their supposed role in the development of reduced GFR in humans.

Introduction

Much effort has been dedicated to the analysis of the cause of acute renal failure. Since clinical investigation has distinct limitations, a large variety of experimental models of acute renal failure have been developed in animals to gain insight into this problem. Nevertheless, the pathogenic principle which makes the kidney curtail filtrate formation and urine production is still subject to controversy [1].

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Andreucci has recently reviewed the divergent causes postulated, such as tubular necrosis, tubulorrhexis, interstitial oedema, debris and cast formation and cell swelling, as well as tubuloglomerular feedback operation and renin activation [2]. A straightforward experimental approach well within the scope of Thurau’s theory [3–6] has been reported by Wunderlich et al [7]. These authors found that deionised serum preparations of anuric patients activated tubuloglomerular feedback in the rat kidney. Microperfusion of these sera into the lumen of the macula densa caused a drastic decrease of single nephron GFR. At variance with physiological feedback operation, which is stimulated by anion transport [8,9] and can be inhibited by furosemide [10,11] this effect was not reversible by simultaneously applied diuretic. The authors concluded that substances other than electrolytes are present in the serum of patients with acute renal failure, which might play an important role in the development of anuria in humans.

In order to verify this notion, we have reproduced the experiments and are pleased to confirm the findings as far as stop flow pressure changes are concerned.

Material and Methods

Serum Preparation

Serum samples were obtained from surgical patients with post-operative acute renal failure, a pre- or post-renal cause having been excluded. A synopsis of the individual ARF patients with positive feedback factors will be the subject of a forthcoming communication. Briefly, 4 patients had multiple aorto-coronary bypass operations, one patient underwent mitral valve surgery, 3 underwent abdominal surgery and one patient had multiple injuries from a traffic accident. All patients developed serum creatinine concentrations exceeding 600 μM/L and had U/P creatinine ratios less than 10 in the course of the ARF. Five patients died from further complications: pulmonary infarction (1), septicaemia (2) and cardiac failure (3). Three patients with polyuric ARF (furosemide treatment) and one with oliguric ARF survived. Serum samples were drawn during the initial two weeks postoperatively. Control sera were obtained from healthy members of the medical staff, from patients with post-operative regular renal function, and from patients with terminal renal diseases receiving regular dialysis treatment (½ to 4 years). The serum samples were prepared for micropuncture by: dialysis against 0.3 M mannitol solutions in cuprophan tubes, staining with 0.1% Lissamine green and centrifugation. The final electrolyte concentrations were determined as 6 to 11 mEq/L Na and below 1 mEq/L Cl and K (flame photometrically: Na and K, colourimetrically: Cl). For analysing the mode of action of the deionised serum (DS) preparations on the feedback sensor at the macula densa, serum samples eliciting a significant SFP decrease were selected for the following experimental groups:

1. DS + 1–3 x 10^-3 M furosemide (Lasix ©, Hoechst, Germany)
2. DS + 1–5 x 10^-3 M theophylline (Solasin ©, Casella-Riedel, Germany)
3. DS perfusion during simultaneous intravenous infusion of verapamil (Isoptin ©, Knoll, Germany, 0.04 mg/min/kg b.w.).
In order to characterise the nature of the non-ionic serum constituents that elicit a feedback response, the different sera were subjected to the following procedures: 1. increased dialysis time up to 24 hr – same protocol as above; 2. protein separation into the three main MW fractions by chromatography (Sephadex Gel 200); 3. dilution with mannitol solution and stirred ultrafiltration (Diaflo ® membranes UM20, Amicon) to the original volume; 4. incubation with acrylic hydrogel coated charcoal (absorbent of Haemocol ® cartridge, Fresenius, Germany).

**Micropuncture Experiments**

Male albino Wistar rats (Han-WIST) of between 180 and 225 g b.w. kept on Altromin ® (Altrogge, Lage, Germany) and tap water were deprived of food 12–14 hr prior to micropuncture experiments. Anaesthesia was performed with Inactin ® (12 mg/100 g b.w., Promonta, Hamburg, Germany). The preparation for micropuncture experiments was as described elsewhere in these proceedings [11]. Micropuncture experiments were performed on randomly selected cortical nephrons according to the protocol depicted in Figure 1.

![Figure 1. Micropuncture protocol of simultaneous SFP monitoring and loop perfusion.](image)

Pressure transmission is effectively prevented by a solid paraffin wax block in between the puncture sites of the pressure and the perfusion pipette.
proximal tubule was blocked with solid paraffin at a mid-proximal site [12]. Two further capillaries were introduced into the pre- and post-blockade nephron segments. Early proximal SFP was monitored using a servo nulling pressure measuring device (Mod.900 micropressure system, WP Instruments, New Haven, Conn) [13]. The loop of Henle was perfused in an orthograde direction with the DS preparations as described above at zero, 10 or 40 nl/min by a Hampel microperfusion pump (pressure dome version, Hampel, Frankfurt, Germany). The microcapillaries used were pulled from Pyrex ® capillary tubing (o.d. 0.9 mm) and the tips were sharpened to o.d. of 3–4 and 7–10 μm for pressure measurements and for microperfusion respectively. The pressure capillary was filled with 0.3% Lissamine green stained 3M NaCl solution. The magnitude of SFP changes was measured in mmHg and is expressed for each of the experimental groups as mean ± SD. The significance of difference was tested using the t-test for unrelated samples and was considered to apply when the two tailed probability did not exceed 0.05.

Results

Representative recordings of early proximal SFP monitoring during loop perfusion with DS are displayed in Figures 2 and 3. The effect of ARF DS (2nd day post operatively) is shown in the upper panel of Figure 2. Orthograde loop perfusion at 40 nl/min resulted in a SFP decrease of between 8 and 12 mmHg in this particular measurement. In contrast to electrolyte perfusates, ARF DS also caused a pressure drop when the perfusion rate was only 10 nl/min, a flow rate well below the normal range (15–30 nl/min). These effects were consistently reversed after stopping perfusion. In contrast to ARF DS loop perfusion, control DS were without significant influence on the feedback sensor as shown in the middle panel of Figure 2. Corresponding results were obtained in selected ARF and control DS respectively. Occasionally effects on the SFP by control DS, which never exceeded 5 mmHg, were promptly relieved by simultaneously perfused furosemide. In contrast to this observation and the evidence of perfusion studies using electrolyte solutions [10,11], the large effect of ARF DS on the feedback sensor was not affected even by high concentrations of furosemide (up to 3 mM), as shown in the upper panel of Figure 3. The mean decrease caused by ARF DS between the 3rd and 7th day after surgery was 12.8 ± 3.6 mmHg.

As a means of removing the causal agents from ARF DS, equilibration with acrylic hydrogel coated charcoal proved highly effective. Respective recordings with the same ARF DS preparation with and without charcoal incubation are demonstrated in the lower panel of Figure 2. Thus the feedback agents can apparently penetrate the acrylic hydrogel membrane, which sieves molecules approximately at m.w. 15,000. In vivo, the ARF DS effect on SFP could be inhibited by simultaneous theophylline perfusion (Figure 3, middle panel) or by i.v. infusion of verapamil (Figure 3, lower panel). Corresponding results were also obtained with other positive ARF DS.

In order to characterise the agent responsible for the ARF DS effect on
Figure 2. Reproduction of original recordings of SFP modulation by loop of Henle perfusion with different serum preparations (see text). The numbers within the thin lined margins signify the loop perfusion rates with the respective DS in nl/min.
Figure 3. Effects of furosemide (upper panel), theophylline (middle) and of verapamil (lower panel) on the ARF DS induced feedback stimulation. For comments, see text.
the feedback sensor, dialysis time against isosmotic mannitol was increased up to 24 hr, which did not abolish its specific effect. One ARF DS preparation was submitted to sephadex chromatography (Sephadex gel 200). After the three main fractions were reconcentrated by stirred ultrafiltration to the initial volumes, the products were dialysed again for 1 hr to remove the electrolytes. A positive feedback effect was then only elicited by fraction 3 with m.w. < 100,000.

Discussion

The present experiments confirm the observations of Wunderlich et al concerning the presence of substances in deionised sera of certain patients with ARF, which cause a drastic SFP decrease, and hence single nephron GFR decrease, by tubuloglomerular feedback stimulation [7]. In agreement with their findings this effect could not be inhibited by furosemide, although considerably higher concentrations were used in our experiments. This apparent furosemide independence was a valuable tool to differentiate reliably between the occasionally obtained small 'false positive' effects by a few control DS, and those of the ARF DS type. Thus the feedback response elicited by the ARF DS must be triggered somehow independently of the physiological receptor. This receptor is said to be chloride ion transport sensitive, since feedback can be prevented by furosemide perfusion, and also by other loop diuretics [10, 11]. ARF DS feedback stimulation was not an irreversible process, as documented by restitution of SFP after stopping perfusion. That at least a part of the feedback signal transmission triggered by ARF DS depends on the physiological pathway is shown by the verapamil, as well as the theophylline effects [14,15]. Concerning the nature of the active agent in ARF DS, our experiments so far hardly justify any statements, but one is tempted to suggest a protein molecule. This, however, would be of little importance in the development of ARF, since being filtered by the glomerulus, it would be rapidly broken down within the first portion of the proximal tubule. Therefore it probably enters the tubule at some later part, possibly from the tubular cells? To elucidate the pathophysiological role of the ARF DS phenomenon, more evidence is necessary than is available so far.

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Open Discussion

BROWN (London) Have you looked for the stuff in the urine of patients with acute renal failure?

GUTSCHÉ We have performed an investigation on the urine of some ARF patients. When the urine was concentrated tenfold by ultrafiltration and the remnant was dialysed to eliminate electrolytes, this solution was also able to lower stop flow pressure as did corresponding sera. Thus we suppose that the substances also occur in the urine in some forms of ARF. But whether the substances are filtered by the glomerulus must remain open.

HAAPANEN (Helsinki) I just wonder whether the pathogenetic factor could be endotoxin. It has been shown that about 40% of patients with acute renal failure have endoxinaemia and endotoxin can cause vasoconstriction.

GUTSCHÉ We are very interested in this question. However, we have not yet performed experiments with suitable endotoxin preparations. At the moment we cannot speculate on whether the ARF serum effect is caused by endotoxins or not.

McGGEOWN (Belfast) The observation that this factor is removed by charcoal suggests the possibility that this might be used as a therapy for acute renal failure. Have you tried charcoal haemoperfusion in your patients and then put their serum through your preparation?

GUTSCHÉ This is a straightforward question. However, as long as we have no direct evidence that these substances are involved with the development of acute renal failure, we would hesitate to use this form of treatment since one may eventually encounter new complications.

RITZ (Heidelberg) I may have missed this in your presentation but did you
check whether the substance you are dealing with is adenosine? You are probably aware of the work in Dr Össwald's laboratory in Aachen. We cannot trace if adenosine, which has a potent vasoconstrictor effect, is generated in acute renal failure. Maybe it is involved in the pathogenesis of acute renal failure. Do you have any information on this?

GUTSCHE Össwald and co-workers (Pflügers Arch., 362, R10, 1976) suggested that adenosine might be involved with ARF. We used theophylline, which is a potent adenosine antagonist in the kidney, and as we have demonstrated, it relieved the feedback effect of ARF DS. However, adenosine is a fairly small molecule (M.wt 267) and it should be dialysed off from the ARF serum after 24 hours. I do not think that 'small' molecular substances could be the causal agents.

RITZ Can the ARF DS effect be safely antagonised by saralasin?

GUTSCHE Schnermann and co-workers (Fed. Proc., 33, 333, 1974) reported that feedback operation was gradually impaired after administration of the angiotensin II analogue. We have not tried saralasin yet. Since we recently demonstrated that renin is not the prerequisite for feedback transmission (Müller-Suur et al (1975) Pfü"gers Arch., 359, 33), saralasin was not the antagonist of choice.