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Original article

HORMONAL DERANGEMENT AND ABNORMAL RENAL HAEMODYNAMICS IN THE ACUTE PHASE OF SEVERE BURNS IN RATS

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A severe burn is characterized by the development of hyperenzymatic levels in plasma and biochemical changes in the blood as well as generalized hormonal dysregulation. This hypercatabolic state reflects the generalized enhanced proteolytic enzyme systems following severe burns. Since anuria often develops in the first hour following severe burns, we investigated the role of renal vasoactive peptides in the early events following severe scalding in order to know the extent of their involvement in the pathogenesis of deranged renal function. Male Wistar rats weighing 200 to 300 g were subjected to a 25% surface area burn by immersing their shaved dorsal surface in water at 70°C for 60 seconds under pentobarbital anaesthesia. A reduction in urinary kallikrein excretion was found (P<0.05) while the renal cortex kallikrein activity remained normal one hour after scalding. An increase in plasma renin activity (P<0.05) and a marked increase in plasma beta-endorphin concentration (P<0.005) was observed. Blood pressure was reduced in the experimental group (P<0.05). Hematocrit levels were elevated (P<0.001) and haemoglobinuria was evident in the first one hour. Plasma proteins remained unchanged. A significant reduction of urine volume was observed in the first hour (P<0.01). Plasma potassium was elevated (P<0.001) and its urinary excretion was reduced (P<0.01). The high plasma renin activity and the enhanced plasma concentration of beta-endorphin suggest that in the first hour following severe burns, hormonal derangements are mainly responsible for abnormal renal hemodynamics.

Key words: *burns-kallikrein-renin-angiotensinogen-kininogen-beta-endorphin*

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INTRODUCTION:

It is known for several years that severe burns cause significant disturbance of renal function (Lindquist *et al*., 1984; Griffiths *et al*., 1983; Haugan *et al*., 1986; Carvajal *et al*., 1976; Ericsson and Rammer, 1972). A marked reduction in glomerular filtration rate (GFR) leads to anuria and acute renal failure (Carvajal *et al*., 1976). Depression of cardiovascular functions following burns has been widely reported (Carvajal *et al*., 1976; Chapman & Speakman, 1988). The hypoproteinemia following loss of plasma proteins through the burned area and the generalized increase in plasma protein breakdown are supposed to worsen the derangement in renal glomerular and tubular functions (Clark *et al*., 1984; Rund *et al*., 1984; Adams *et al*.,1986).

Plasma kallikrein and kinins and its components has been extensively studied in burns (Rund *et al*., 1984; Adams *et al*., 1986). However the involvement of renal kallikrein in the regulation of kidney function in burn-shock and in the face of wide spread hormonal changes especially in the early phase of severe burns remains controversial. The significance of increased plasma endogenous morphine-like peptide, beta-endorphin, variously reported following burns (Osgood *et al*., 1987; Dietch *et al*., 1988), in the regulation of renal function is undefined. Even then, the systemic enzymes and hormonal derangement following burns has been reported to involve a wide number of organs like the heart (Chapman and Speakman, 1988; Hilton *et al*., 1987), liver (Shires *et al*., 1983), pancreas (Mauench *et al*., 1987), gastrointestinal tracts and lungs (Clark *et al*., 1984). In addition, altered muscle glucose metabolism (Clark *et al*., 1984) and biochemical and functional alterations in macrophages (Loose *et al*., 1984) has been reported in burns.

The hormonal changes which may alter renal function as well as the patho-physological mechanisms by which the kidney respond to these changes in severe burns, require further clarification. To assess the possible participation of vasoactive peptides in the early events following burns and in the pathogenesis of the ensuing renal function derangements, we investigated the involvement and possible interaction of renal vasoactive hormonal systems like the renin-angiotensin system, renal kallikrein-kinin system and beta-endorphins in the first hour following severe burns in rats.

MATERIALS AND METHODS:

Animal preparation:

Male Wistar rats weighing 200-300 gms, allowed free access to, tap water and normal rat-chaw (Altromin, Lage, Germany) were placed in

separate cages 2 days before the experiment. The room in which the animals were kept had a temperature of 28°C and a relative humidity of 60%; both were kept constant using an electronic device. On the day of the experiment, the rats were anaestesized with sodium pentobarbital (Nembutal ^{R}, 40mg/kg body weight) intraperitoneally. A polyethylene catheter (PP50) was placed in the femoral artery for direct blood pressure measurement and for the collection of blood samples. Another catheter (PP50) was placed in the urinary bladder for urine collection. The dorsal surface of the rats was shaved of hair using electric clippers. The shaved area was measured by planimetry. Earlier experiments revealed that 20% surface area burns in S.D. rats of similar size lead to anuria which remained for about 7 hours following burns (Loew *et al*., 1974). From the relationship between surface area (S) and body weight (G), a constant according to Rubner's formular (S=GK; where K=constant) was obtained (Rubner M. (1883): Z Biol. 19:535) and from this, the body surface area was calculated. In order to obtain a standardized surface area burn, blocks of plexy-glass were constructed to the same contour of the rat's back. Through an opening in the plexy-glass, the area to be burned was immersed in water at 70°C for 60 seconds. Control rats were treated similarly but with tepid water. Based on a pilot experiment, the area to be burned was chosen to comprise 20 to 25 % of total body surface area. Blood pressure was recorded before and one hour after scalding. Blood samples were collected from the control and experimental rats one hour after scalding. Similarly, urine samples were collected during the two periods of 30 min. each for one hour after burns. The animals were kept under anaesthesia throughout the experiment.

At the end of the experiment, kidneys were decapsulated in-vivo and excised. Pieces of renal cortexes weighing 10 to 20 mg were homogenized in desoxycholate and centrifuged for 30 minutes at 50,000 g. Kallikrein was determined in the supernatant as previously described (Bönner & Marin-Grez, 1981).

Analytical methods: Urine volume was determined gravimetrically in pre-weighed Eppendorf vials. Plasma sodium and potassium as well as urinary concentration of the electrolytes were measured using internal standard flame photometry. Kallikrein was measured in urine and kidney homogenates by incubating appropriately diluted urine samples and supernatant of kidney homogenates with synthentic D-Val-Leu-Arg-p-Nitroanilide (S2266 Kabi substrate, Kabi Diagnostics GmBH, Munich, Germany) at 37°C for 120 min as previously reported (Bönner & Marin-Grez, 1981). Blanks did not contain the substrate. For the measurement of kininogen, one part of plasma was diluted in 19 parts of Tris-Hcl-buffer (0.1M; pH8.5) and boiled for 30 minutes. Trypsin, 0.2 mg/ml (end concentration) was added at 37°C. After a 60 minutes incubation period, the reaction was stopped with 99% ethanol (40 X vol). The solution was then dried at 70 °C under vacuum (13.3 Pa.). The released kinins was measured by radioimmunoassay as previously reported (Marin-Grez *et al*., 1974). The kininogen concentration was expressed in ng bradykinin equivalent per ml.

Beta-endorphin was measured by radioimmunoassay as follows: Antibody was obtained by coupling human beta-endorphin by the carbodiimide method to human thyroglobulin and injecting this antigen intradermally into male New Zealand rabbits every other day for 10 days. The last injection was given 6 weeks thereafter. After 10 more days, blood was collected and the sera was used for the assay. Plasma beta-endorphin was adsorbed onto octadecasilyl silica cartridges (Sep-Pak ^{R}). After rinsing, the adsorbed beta-endorphins were eluated with isopropyl alcohol. The eluate was dried at room temperature under vacuum (13.3 Pa.). After dissolving the end product in sodium-potassium -phosphate buffer (0.05 M; pH7.6; 0.1% Haemacel; 0.1% Triton^{-X100}), it was incubated at 4 °C with ^{131} I-labelled beta-endorphin and antisera for 24 hours. The tracer was labeled using the Chloramin-T method (Greenwood *et al*., 1963). Bound and unbound tracer was separated using dextran-coated charcoal.

Plasma renin activity was measured by incubating plasma with partially purified rat angiotensinogen. The released angiotensin-I was measured by radioimmunoassay (Ganten *et al*., 1980). Angiotensinogen was also measured by radioimmunoassay of angiotensin-I after its release following incubation with excess of Hog renin as described previously (Ganten *et al*., 1980).

Plasma protein was determined using the method of Lowry *et al* (Lowry *et al*., 1951). Serum-alpha-amylase was determined according to Zulkowsky (Bernfield, 1955). Maltose (Serva, Heidelberg Germany) was used as standard. Haematocrit was determined by micro-capillary centrifugation. Haemoglobin in urine was determined by adapting the benzidine reaction for quantitative measurements (Marin-Grez *et al*., 1982).

Statistical Analysis: The significance of difference between groups was tested with the "t" test of student as appropriate. Paired "t" test was used for comparism of data obtained from the same group of animals while unpaired "t" test was used between experimental and control group. Values are expressed as means ± SEM (standard error of means). P value less than 0.05 was accepted as statistically significant.

RESULTS

One hour after 25% surface area burn (70 °C for 1 min.), there was a reduction of blood pressure in the experimental group (88 ± 5 mmHg vs. 108 ± 2.4 mmHg n=8; P<0.05), fig 1. The plasma potassium concentration was increased (4.4 + 0.1 mmol/L vs. 3.6 ± 0.1 mmol/L; n=8; P<0.001) fig 1. There was no change in plasma sodium concentration (fig 1). An increase in haematocrit was found (47.5 ± 0.8 vs. 41.9 ± 0.6%, n=8, P<0.001) fig 2. An enhanced plasma renin concentration was observed (P<0.05, fig 2).

Fig. 1.
Effect of 25% surface area burn on arterial blood pressure (BP) before and after burns and plasma concentration of sodium [Na^+] and potassium [K^+] in control and experimental Wistar rats. Initial = before burns; Final = 1 hour after burns.

Fig. 2. Effect of 25% surface area burn on haematocrit (HT) and plasma renin concentration (PRC) in Wistar rats.

Fig. 3. Effect of scalding (25% body surface area) on plasma proteins, angiotensinogen, kininogen, and alpha-amylase activity in plasma of Wistar rats

There was no change in plasma protein concentration as well as in plasma total kininogen

and plasma angiotensinogen concentration (fig.3). Serum alpha-amylase activity showed no difference with respect to control period (fig 3). Beta-endorphin concentration in plasma on the other hand, showed a marked increase one hour after scalding (112 ± 23 vs. 56 ± 10 pg/ml, n=8; P<0.005) fig 4.

Urinary kallikrein excretion was reduced 30 to 60 minutes after thermal injury (P<0.05) fig 5. There was no change in renal cortex kallikrein activity (55 ± 10 vs. 53 ± 8 mU/g kidney; n=8; p=Not Significant). Hemoglobinuria was evident. Haemoglobin was not detectable in the urine of control rats while it rose to 311 ± 96 µg/min in the 0 to 30 min period and to 144 ± 64 µg/min in the 30 to 60 min period after scalding. A reduction in urine volume was observed in the 30 to 60 min period after severe burns (57.7 ± 7.7 µl vs. 100 ± 11.54 µl, n=8; P<0.01). A reduction in urinary excretion of potassium was also observed (7.23 ± 0.87 vs. 13.87 ± 1.88 µmol/l; P<0.01) fig 6. No significant change in the urinary excretion of sodium was observed. (Fig. 6).

Fig. 4. Effect of scalding on plasma concentration of beta-endorphin in Wistar rats subjected to a 25% body surface area burns.

Fig. 5. Effect of 25% body surface area burns on renal kallikrein excretion in Wistar rats.

Fig. 6. Effect of severe burns on urine flow rate (V), renal sodium excretion (Na^+) and renal potassium excretion (K^+) in Wistar rats

DISCUSSION:

Severe burn is characterized by the development of hyperenzymic levels and biochemical changes in the blood as well as generalized hormonal dysregulation (Clark *et al*., 1984; Rund *et al*., 1984; Loose *et al*., 1984; Balogh *et al*., 1984). This hypercatabolic state reflects the generally enhanced proteolytic enzyme systems following severe burns (Rund *et al*., 1984; Adams *et al*., 1986). In the presence of greater than 25% surface area burns, major changes in systemic and renal hemodynamics occur (Haugan & Kirkibo, 1986, Chapman & Speakman, 1988). There is a significant reduction in blood volume, decrease in cardiac output and an increase in total peripheral resistance (Carvajal *et al*., 1976; Hilton *et al*., 1987, Rammer, 1973). Severe burn is commonly associated with reduction in urine flow as observed in this study. Oliguria may be explained by a reduction in GFR and an increase in tubular fluid reabsorption as a result of decreased renal blood flow secondary to volume depletion and hormonal disturbances (Loew & Meng, 1974; Carvajal *et al*., 1976)

Our finding of a reduced blood pressure and a raised haematocrit in the face of a normal plasma protein concentration in the first hour suggest that

systemic haemodynamic disturbance (Carvajal *et al*., 1976; Chapman & Speakman, 1988) is an important factor leading to disturbed renal haemodynamics. The decrease in cardiac output and circulatory blood volume may be connected to the high plasma vasopressin levels, which has been reported following thermal injury (Hilton *et al*., 1987). However, the derangement in renal blood flow is thought by others to be mediated via serotonin and thromboxane A2 receptors since blocking of these receptors with kentanserin improved renal blood flow (Huangan & Kirkebo, 1986); an effect that could also be observed with angiotensin II receptor blockade using saralasin and adrenergic blockade with phentolamin which also improved renal blood flow to some degree (Huagan & Kirkebo, 1986). Vasopressin could induce coronary vascular constriction and myocardial ischaemia as well as increased peripheral resistance. These effects can be blocked with vasopressin V1 receptor antagonist (Hilton *et al*., 1987). The high plasma concentration of vasopressin could also lead to increased tubular fluid reabsorption further reducing urine flow.

Our finding of a raised plasma renin concentration and as reported by other workers (Griffiths *et al*., 1983) in the first hour following severe burns suggest that systemic effects of the renin-angiotensin system through its vasoconstrictive effects could reduce renal blood flow. Intrarenal production of renin could lead to local production of angiotensin II. Since angiotensin II has been shown to preferentially constrict the efferent arterioles (Edwards, 1983), this would tend to prevent a marked fall in filtration pressure.

Following severe burns; plasma kininogen is rapidly broken down (Adams *et al*., 1986; Bönner *et al*., 1988). A decrease in low molecular weight kininogen and kininase II has been reported in patients after thermal injury (Bönner *et al*., 1988). It is likely that a rise of T-Kininogen, as an acute phase inflammatory protein, obscured a fall in low molecular weight kininogen in our acute experiment since we measured total kininogen.

Kinins together with other inflammatory agents like histamine could be responsible for increased vascular permeability. The finding of a reduced kininase II activity and enhanced kinin activity in plasma suggests that kinins are important mediators of hypotension following thermal injury.

In conformity with previous reports (Bönner *et al*., 1988), we found reduced kallikrein excretion even in the first hour following scalding. Renal kallikrein Kinin system has been suggested to be involved in renal excretion of water and electrolytes (Odigie & Marin-Grez, 2000; Carretero & Scicli, 1976; Mills *et al*., 1976). Thus decreased activity of the renal kallikrein system, perhaps by reduced release of kinins, could contribute to renal vasoconstriction and oliguria. Furthermore, reduced renal kallikrein release could reduce the protective effects of renal kallikrein on renal vasculature against the constrictor effects of the renin-angiotensin system.

Proximal tubular dysfunction, in the form of enhanced proteins and substrate clearance has been reported following burns (Linquist *et al*., 1984). Thus a reduction in renal kallikrein excretion can also be explained by enhanced filtration and excretion of kallikrein inhibitors. The possibility that like the proximal tubular disturbance, a reversible distal tubular abnormality is responsible for the reduction of urinary kallikrein excretion cannot be disregarded. Studies on distal tubular function following thermal injury should clarify this possibility.

Beta-endorphin levels in plasma increase rapidly after burns as reported herein and by other workers (Osgood *et al*., 1987). Beta-endorphins is not only known to modify the response to pains (Osgood *et al*., 1987) but is also increased in shock situations (Elliot *et al*., 1985). This peptide has been implicated in complex cardiovascular changes leading to hypotension (Elliot *et al*., 1985). However, beta-endorphins are not invariably increased in all stress situations: it is not increased in unstable angina pectoris but increased in acute myocardial ischaemia even though the pains involved in both conditions are comparable (Bach *et al*., 1987). Increase in beta-endorphin is more marked in conditions associated with enhancement of the renin-angiotensin-system activity so that beta-endorphins could prevent a reduction of cerebral blood flow by antagonizing the vasoconstrictory effects of the renin angiotensin system on cerebral vasculature. This is in agreement with previous observation showing that beta-endorphins block the cerebral pressor action of angiotensin II (Innanen *et al*., 1987). Although beta endorphins has been shown to bind to peripheral opiate receptors in the kidney and stimulate the cyclic AMP-adenosine monophosphate system (Dave *et al*., 1985), this in-vitro observation may not be physiologically important for renal function in burn-shock. This is further supported by the fact that the plasma concentration of beta-endorphin tends to normalize after about 2 days (Osgood *et al*., 1987) and is therefore not increased in the chronic state. This suggests that its action may be beneficial only in the acute phase of severe burns.

Although the clearance of amylase has been reported to be increased following thermal injury (Muench *et al*., 1987; Linquist *et al*., 1984), our finding of a constant plasma amylase level in the first hour following severe burns suggest that such an effect does not lead to a fast amylase accumulation. In addition, the present findings also suggest that involvement of organs like the pancreas and salivary glands is not a primary event in the humoral disturbances observed after severe burns.

We conclude that in the first hour following severe burns, humoral derangements are mostly responsible for abnormal renal haemodynamics and that the observed effects of burns on renal function disturbance in the acute phase, like in the established phase of severe burns, is of multifactorial etiology.

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