Abstract. Tubuloglomerular feedback (TGF) function and autoregulation (renal blood flow RBF; glomerular filtration rate, GFR; single-nephron glomerular filtration rate, SNGFR) were examined in rats chronically treated with deoxycorticosterone acetate (DOCA) and given isotonic saline to drink. DOCA treatment depressed arterial plasma renin activity, expanded plasma volume by 25% and increased arterial blood pressure. Autoregulation of RBF and GFR was maintained in the DOCA animals above 90 mm Hg and 110 mm Hg respectively, whereby both GFR and RBF were lower than in controls. Micropuncture experiments demonstrated the absence of TGF in the DOCA animals. There was no difference between SNGFR values measured in the distal and proximal tubules, nor was there a significant response of SNGFR when loops of Henle were perfused with Ringer's solution at 20 nl/min. Loop perfusion in control rats with tubular fluid collected in DOCA rats elicited a normal TGF response, showing that TGF inhibition in the DOCA animals is due to changes in the function of the juxtaglomerular apparatus. In contrast to control rats, proximal SNGFR was perfectly autoregulated. These results suggest that TGF is not primarily responsible for autoregulation and that the vasodilatation normally resulting from acute TGF interruption is therefore compensated by some other mechanism such that RBF and GFR are lower than in controls.

Key words: Autoregulation - Tubuloglomerular feedback - Renal blood flow - Glomerular filtration rate - Plasma renin activity - Deoxycorticosterone acetate - Plasma volume - rats

Introduction

Since the work of Rein [28] and Medes [20, 21] more than 50 years ago, it is known that, within a certain range, variations of systemic blood pressure or renal perfusion pressure are compensated by the kidney by parallel changes in renal vascular flow resistance so that the renal blood flow (RBF) and the glomerular filtration rate (GFR) remain constant (autoregulation). Whilst it is generally agreed that regulatory mechanisms intrinsic to the kidney are responsible for this phenomenon there is still no general agreement on the nature of the mechanism. In particular, debate continues concerning the relative importance of two regulatory mechanisms: (a) a "myogenic" mechanism whereby increased transmural pressure or wall tension directly elicits smooth muscle contraction in the preglomerular renal vasculature [2] and (b) a negative (metabolic) feedback mechanism, the so-called tubuloglomerular feedback mechanism (TGF), in which changes of blood pressure induce parallel changes in the luminal NaCl concentration in the macula densa segment of the nephron. These in turn trigger parallel changes in renal vascular flow resistance [5, 25]. Immediately relevant to this debate are findings from studies on various states of chronic volume expansion. In these states autoregulation of GFR and RBF have been reported to persist (for literature see below) whereas TGF function has been reported to be attenuated or even abolished [8, 22, 23, 34]. This especially holds for the model in which volume expansion is achieved by daily deoxycorticosterone acetate (21-hydroxy-4-pregnene-3,20-dione acetate; DOCA) administration with isotonic saline to drink, for at least 2 weeks. Under these conditions TGF in superficial nephrons is almost completely inhibited [6] whereas RBF and GFR in both rats [1, 17] and dogs [10, 16, 18, 19, 24, 27] appear to remain normally autoregulated. Since under these experimental conditions uncontrolled differences in experimental protocol, as well as inhomogeneity of superficial and subcortical nephron function, cannot be excluded, it was the aim of the present study to examine TGF function and autoregulation of GFR in superficial nephrons in parallel and to relate these data to the autoregulation of RBF and GFR in the whole kidney.

Experiments were performed in rats in which the renal perfusion pressure was experimentally lowered stepwise below the spontaneous pressure and the effect of this on TGF,
paring the rats as above and allowing an equilibration period of about 

Renin activity was determined in arterial plasma in a separate series 

Measurement of renin activity in arterial plasma 

Measurement of plasma volume 

In a separate series of experiments, plasma volume was measured in both groups by the Evan’s blue dilution technique as described elsewhere [6]. 

Measurement of renin activity in arterial plasma 

Renin activity was determined in arterial plasma in a separate series on animals kept on the dietary treatment for 1–6 weeks. After preparing the rats as above and allowing an equilibration period of about 20 min, 200 μl arterial blood was withdrawn from the femoral artery into heparinized haematocrit tubes. After separation the plasma was stored at −30°C (1–3 weeks) until assay.

Micropuncture experiments 

In vivo activity of TGF. Rats of both groups were prepared as above. Following equilibration, the proximal and distal loops of a nephron were identified as described earlier [6]. Using an oil-filled micropipette (outer diameter, 8 μm) a timed, quantitative fluid collection was first made from the distal tubule. Under these circumstances flow at the TGF-sensing site (macula densa) is quasi-normal and, hence, the TGF control loop intact. Subsequently a timed, quantitative collection was made from the proximal tubule. Under these circumstances the TGF control loop is interrupted. The difference between “proximal” and “distal” SNGFR is thus an index of the in vivo activity of the TGF mechanism.

Loop of Henle perfusion studies. “Open-loop” TGF activity was tested in rats of both groups by perfusing loops of Henle with either Ringer’s solution stained with FD&C green, or homologous, late proximal tubular fluid previously harvested in the experimental animal and simultaneously measuring SNGFR from the proximal tubule. This method is described in detail elsewhere [6, 15]. Perfusion rates were 0, 20 and 40 nl/min. In any one nephron at least three perfusion rates, including 0, were tested. Each subsequent collection was made further upstream to avoid the possibility of leakage.

Autoregulation experiments. Rats from both groups were prepared as above. After selecting one of the nominal blood pressures by adjustment of the aortic clamp, a random proximal tubule was punctured and distal loops identified as above. Several distal-proximal paired, quantitative tubular fluid collections were made and the blood pressure was then changed. After a further equilibration period, further sample pairs were collected.

Analyses 

Clearance experiments. Urine volume was determined by weight, assuming a relative density of 1. Inulin concentration in plasma and urine was measured by the anthrone method [13]. Haematocrit was measured in all blood samples. Plasma protein concentration was measured using a commercial kit (Total Protein, Boehringer Mannheim, FRG). Plasma Evan’s blue was measured spectrophotometrically by measuring the difference in plasma absorbances at 610 nm and 670 nm against a plasma blank and comparison with a standard curve also prepared in homologous plasma.

Plasma renin activity. Arterial plasma renin activity (PRA) was measured enzyme-kinetically using the angiotensin I radioimmunoassay technique. Plasma samples (20–60 μl) were diluted with 50 μl 150 mmol l⁻¹ phosphate buffer (pH 6.5) containing peptidase blockers. The mixture was incubated at 37°C for up to 42 min, whereafter 10–20 μl of this incubation mixture was incubated with angiotensin I antibody and radiolabelled angiotensin I in phosphate buffer at pH 7.4 for 2 h. The final antibody dilution was 1:50000. The unbound octapeptide was separated from the antigen-antibody complex on dextran-coated charcoal in phosphate buffer and the concentration of the unbound tracer determined in a gamma counter. The initial slopes of the resulting curves were taken as measures of renin activity. With this method samples could be stored at −30°C for up to 3 weeks with no loss of activity (for details of this method see [30]).

Micropuncture experiments. The volume of the tubular fluid samples was measured by injecting the sample into an oil-filled, constant-bore glass micropipillary (Minicap, 0.5 μl, Hirschmann, Heilbronn-Eberstadt, FRG) and measuring the length of the column by means of an ocular micrometer. Inulin was determined in plasma and urine by perchloric acid hydrolysis to fructose, which was assayed by the hexokinase/glucose-6-phosphate dehydrogenase method [4, 29]. Reagents