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ORIGINAL ARTICLE

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Site of action of moxonidine in the rat nephron

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Abstract Moxonidine is a centrally acting antihypertensive agent which has been found to exert its blood pressure lowering effect by interaction with α_2-adrenoceptors and imidazoline receptors of the I_1-type. These receptors have also been demonstrated to be present in the rat kidney. In the present study, clearance and micropuncture techniques were applied to anaesthetized rats to localize the site of action of moxonidine within the nephron. The clearance data show that moxonidine (0.25 mg/kg i.v., followed by a continuous i.v. infusion of 0.25 mg/h) induced a marked increase in urine flow and urinary excretion of sodium, chloride and potassium. The changes in urine flow and urinary solute excretion were accompanied by an enhanced glomerular filtration rate. The micropuncture experiments revealed that moxonidine significantly increased glomerular filtration rate of superficial nephrons, and significantly inhibited fractional reabsorption of fluid, sodium, potassium and chloride by similar amounts (by 9.0%–9.8%) in superficial proximal tubules. Regarding fluid and sodium reabsorption, the proximal effect of moxonidine was continuously weakened by a compensatory increase of reabsorption in the loop of Henle and the subsequent distal nephron segments. The inhibitory effect of moxonidine on fractional proximal potassium reabsorption was completely compensated in the loop of Henle, but the drug induced a net secretion of potassium into the segments lying beyond the early distal tubule, probably as a consequence of the increased tubule fluid and sodium load delivered to them. The experiments have identified the proximal tubule as the principal nephron site where the diuretic action of moxonidine arises. The proximal effect may be related to the increased glomerular filtration rate and to a direct inhibitory interaction of moxonidine with the proximal Na^+/H^+ exchanger.

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Introduction

Moxonidine is a centrally acting antihypertensive drug which is believed to exert its cellular action by interaction with α_2-adrenoceptors and imidazoline binding sites (Ernsberger et al. 1993, 1997; Ziegler et al. 1996; Head et al. 1997; Bock et al. 1999). The imidazoline binding sites have been suggested to represent a distinct class of non-adrenoceptors. Two subpopulations of imidazoline binding sites have been described up to now. The I_2-site is believed to reside intracellularly on the monoamine oxidase (Tesson and Parini 1995; Gargalidis-Moudanos et al. 1997), a catecholamine-degrading enzyme, whereas the I_1-binding site is located in the plasma membrane (Piletz and Sletten 1993). Moxonidine is the most selective agonist at the I_1-binding site, so far described. It has a 33-fold higher affinity for I_1-binding sites than for α_2-adrenoceptors (Ernsberger et al. 1997).

In a previously published study (Hohage et al. 1997) we demonstrated that moxonidine, in addition to its antihypertensive effect, induces a marked diuresis and saluresis when injected intravenously into anaesthetized rats. Also intrarenal (Allan et al. 1993) or intracerebro-ventricular (Penner and Smyth 1994) administration of moxonidine resulted in an increase in sodium and fluid excretion in unilaterally nephrectomized rats, indicating that the diuresis may arise from both central and renal effects of this drug.

In the present study we employed micropuncture techniques to anaesthetized rats to localize the site of diuretic action of moxonidine within the nephron. A preliminary account of the present work was given previously (Greven and von Bronewski-Schwarzer 1999).

Materials and methods

All experiments were performed on male Sprague-Dawley rats. The animals were bred in the „Zentrallaboratorium für Versuchs-

tiere, RWTH Aachen“ and were placed in a room with the lighting adjusted to produce a normal day/night cycle (illuminated from 8 h to 20 h). The animals were maintained on a standard rat chow (Altromin standard diet) and were allowed ad libitum access to water and food until the beginning of the experiments. All animals were anaesthetized by intraperitoneal injection of sodium thiobutabarbital (Inactin, 100 mg/kg b.w.), and subsequently tracheotomized. The left jugular vein was cannulated for infusion and drug application. The urinary bladder was exposed by an abdominal incision and cannulated to drain urine. The urethra was ligated.

The micropuncture technique applied to these animals was identical to that previously published (Greven 1995). In short, the left kidney was exposed through an abdominal incision. The kidney was fixed in a lucid holder and bathed with mineral oil at 37°C. The renal capsule was not removed. During the experiments the animals were placed on a heated table and rectal temperature was maintained at 37°C. After the surgical procedure, 2 ml of isotonic saline was injected intravenously. The animals (mean body weight of 255 g, range 240–275 g) then received a priming dose of 200 μCi [^{3}H]inulin, dissolved in 1 ml of isotonic saline. Subsequently, 200 μCi [^{3}H]inulin, dissolved in isotonic saline, were infused intravenously at a rate of 4.5 ml/h. The experiments began 30 min after the start of the infusion. After 2–4 control punctures, performed during a time period of 30 min, moxonidine was injected and infused intravenously, and 2–4 additional punctures were performed during a time period of 40 min in each rat. Late proximal and early distal tubular segments were identified by intravenous injection of lissamine green (0.01 ml of a 10% solution, buffered to pH 7.0). The intratubular flow rate was estimated by timed collection of the tubular fluid. Collecting time of the tubular fluid usually was 5 min. The rate of collection was adjusted to maintain the position of a large drop of castor oil placed distal to the site of puncture. Particular care was taken not to alter the diameter of the tubule. In each of the rats, samples of blood and urine of the bladder were obtained for measurements of glomerular filtration rate, urine flow and urinary excretion of sodium, chloride and potassium. Previous micropuncture studies from this laboratory, where the left ureter was cannulated in addition to the urinary bladder, have established that the function of the left micropunctured kidney did not differ from that of the right untouched kidney under control conditions and after injection of a diuretic drug (ozolinone; Greven et al. 1978).

Nephron filtration rate was calculated from tubular flow rate and tubular fluid to plasma inulin ratio. Fractional reabsorption rates were calculated according to customary formula.

The ^{3}H activity in plasma, urine and tubular fluid was determined by standard isotope techniques. The chloride concentration in the tubular fluid was determined by electrometric titration, sodium and potassium using an ultramicroflame photometer (Hampel, Frankfurt, Germany). The volume of the collected tubular fluid was measured in a constant bore glass capillary previously calibrated with a radioactive standard solution. The same capillary was used for all measurements.

Drugs. The following drugs were used in this study: [^{3}H]inulin (specific activity 5 Ci/mmol; Amersham Buchler, Braunschweig, Germany), sodium thiobutabarbital (Inactin; Byk-Gulden, Konstanz, Germany), moxonidine (Beiersdorf-Lilly, Hamburg, Germany). All other chemicals were purchased from Sigma Chemical (Darmstadt, Germany) at the highest purity available.

[^{3}H]Inulin and thiobutabarbital were dissolved in isotonic saline. The moxonidine solution was prepared by dissolving 100 mg of the drug in 90 ml of isotonic saline, containing 1 ml of 1 N HCl. Subsequently, 10 ml of isotonic saline containing 1 ml of 1 N NaOH was added. Immediately before starting the experiment, the solution was diluted 1:4 with isotonic saline.

Statistics. All data are given as means ± SEM. For statistical evaluation, the values measured during the control periods and the periods after moxonidine were averaged, and the differences were analyzed using Student's *t*-test. The differences were considered to be significant at a level of $P<0.05$.

Results

The effect of moxonidine on overall kidney function, as measured during the micropuncture experiments, is depicted in Table 1. The drug was injected intravenously at a dose of 0.25 mg/kg, followed by a continuous intravenous infusion of 0.25 mg/h. Moxonidine induced a marked and significant ($P<0.01$) increase in urine flow and urinary excretion of sodium, chloride and potassium. The changes in urine volume and urinary solute excretion were accompanied by a significant ($P<0.01$) increase in the glomerular filtration rate. The changes of kidney function could be observed immediately after application of the drug and, due to the continuous infusion of moxonidine, persisted as long as the experiments lasted. Control experiments in the absence of moxonidine ($n=10$) showed no significant time-dependent changes of the parameters of kidney function depicted in Table 1.

In Table 2, the results obtained by micropuncture of superficial late proximal tubules are summarized. Moxonidine induced a significant increase of the single nephron glomerular fitration rate, and of the flow rate of the tubular fluid. The tubular fluid to plasma inulin concentration ratio decreased significantly, whereas the sodium, chloride and potassium concentrations in the tubular fluid remained unchanged.

Table 1 Clearance data in micropuncture experiments ($n=10$). Moxonidine was injected and infused intravenously after three control measurements performed during a time period of 30 min. All of the moxonidine-induced changes were statistically significant

Time (min)	GFR (ml/min × 100 g b.w.)	Urine flow (μl/min × 100 g b.w.)	Urinary excretion (μmol/min × 100 g b.w.)			Fractional urinary excretion (%)			
			Na	Cl	K	Na	Cl	K	H_2O
0–10	1.18±0.24	2.28±0.32	0.21±0.06	0.65±0.09	0.42±0.06	0.10±0.04	0.42±0.07	6.98±1.09	0.15±0.03
10–20	1.01±0.18	2.28±0.36	0.23±0.07	0.68±0.12	0.43±0.09	0.13±0.06	0.50±0.09	7.68±1.56	0.17±0.04
20–30	1.02±0.16	3.08±0.57	0.38±0.12	1.01±0.20	0.56±0.13	0.18±0.06	0.71±0.13	9.84±1.72	0.22±0.05
Moxonidine (0.25 mg/kg i.v. and 0.25 mg/h i.v.)									
40–50	1.50±0.20	48.6±9.02	3.06±0.58	5.90±0.61	1.78±0.11	1.43±0.29	3.69±0.41	29.6±1.77	3.03±0.54
50–60	1.13±0.12	67.1±9.84	2.03±0.46	5.05±0.44	1.69±0.08	1.21±0.29	3.93±0.46	34.1±2.88	5.24±0.97
60–70	1.42±0.14	61.0±10.6	2.13±0.54	4.95±0.61	1.74±0.09	1.43±0.41	4.31±0.66	38.7±3.28	5.14±1.00
70–80	1.58±0.16	44.8±7.27	2.24±0.44	5.10±0.68	1.57±0.12	1.39±0.30	4.27±0.71	33.1±2.20	3.44±0.65

Table 2 Summary of micropuncture data obtained by late proximal collections (*SNGFR* single nephron filtration rate, *TF* tubular fluid, *TF/P* tubular fluid/plasma ratio, *n.s.* not statistically significant)

	Control (n=5)	Moxonidine (0.25 mg/kg i.v. and 0.25 mg/h i.v., n=5)
SNGFR (nl/min)	40.9±3.59	48.1±3.81*
Flow rate (nl/min)	13.1±1.28	20.1±1.87**
TF/P inulin	3.29±0.21	2.52±0.20**
$TF_{Na}+$ (mmol/l)	134 ±5.06	137 ±3.98 n.s.
$TF_{Cl}-$ (mmol/l)	139 ±5.84	139 ±4.55 n.s.
$TF_{K}+$ (mmol/l)	4.69±0.24	4.50±0.13 n.s.

*P<0.05, **P<0.01

Table 3 Summary of micropuncture data obtained by early distal collections. For abbreviations see Table 2

	Control (n=5)	Moxonidine (0.25 mg/kg i.v. and 0.25 mg/h i.v., n=5)
SNGFR (nl/min)	39.4±2.26	50.3±3.58**
Flow rate (nl/min)	5.01±0.56	9.69±0.62**
TF/P inulin	9.41±1.24	5.49±0.35**
$TF_{Na}+$ (mmol/l)	38.0±3.06	40.9±2.02 n.s.
$TF_{Cl}-$ (mmol/l)	38.2±2.69	37.2±1.68 n.s.
$TF_{K}+$ (mmol/l)	2.11±0.41	1.14±0.05*

The values obtained from punctures of early distal tubules are shown in Table 3. Again, the single nephron glomerular filtration rate increased significantly after moxonidine application. Also the flow rate of the tubular fluid was enhanced by moxonidine whereas the tubular fluid/plasma (TF/P) inulin ratio and the potassium concentration in the tubular fluid decreased significantly. The solvent of moxonidine had no significant effects at the late proximal (n=9) or early distal puncture sites (n=9).

From the data of Tables 1, 2 and 3, the fractional tubular reabsorption of fluid, sodium, chloride and potassium, as measured at different nephron sites and in urine, can be calculated. These data are summarized in Fig. 1 and Table 4. It is evident from Fig. 1 that the most marked effect of moxonidine on tubular fluid reabsorption occurred in the proximal tubule. The drug decreased fractional fluid reabsorption by 9.2% in this nephron segment. The proximal effect of moxonidine was partially compensated in the nephron segment, lying between the late proximal and the early distal tubular site, i.e., the loop of Henle. Thus, the effect of moxonidine measured at the early distal tubule (decrease of fractional reabsorption by 7.6%) was smaller than at the late proximal tubule. The effect of moxonidine on overall kidney fractional fluid reabsorption, as derived from the urinary data, was less pronounced (decrease of fractional reabsorption by 4.0%) than at the early distal tubule.

Regarding fractional tubular sodium reabsorption (Table 4), the results obtained were very similar to fluid reabsorption. The moxonidine effect was most marked in the proximal tubule. The proximal effect was continuously diminuted in the subsequent nephron segments.

The effect of moxonidine on fractional chloride reabsorption (Table 4) was identical to fractional sodium reabsorption at the late proximal and the early distal tubules but the moxonidine effect was even slightly more pronounced in urine than at the early distal tubule.

Fractional proximal potassium reabsorption (Table 4) was affected by moxonidine similar to sodium and chloride reabsorption. In contrast to sodium and chloride, moxonidine did not at all affect fractional potassium reabsorption at the early distal tubular site but increased fractional urinary potassium excretion markedly.

Discussion

This investigation has shown that, similar to our previously published study (Hohage et al. 1997), moxonidine

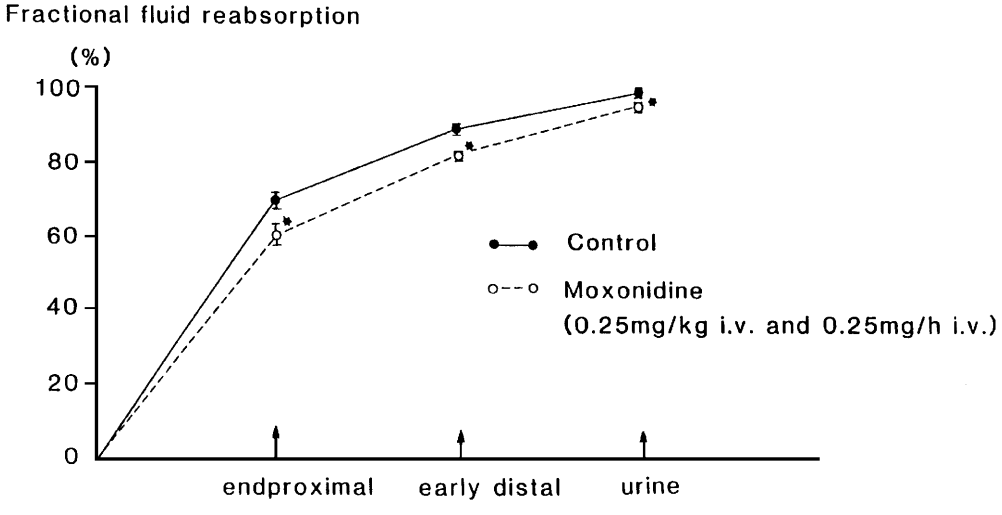

Fig. 1 Effect of moxonidine on fluid reabsorption in the nephron. Superficial nephrons were punctured at different sites (endproximal, n=5, and early distal, n=5). Fluid reabsorption is expressed as a percentage of the glomerular filtration rate (fractional fluid reabsorption). Whole kidney fractional fluid reabsorption is indicated by the values measured in urine (n=10). *P<0.01

Table 4 Fractional tubular reabsorption (per cent reabsorption of filtered load) with control conditions and after moxonidine (0.25 mg/kg i.v., 0.25 mg/h) as measured at different nephron sites and in urine

	Late proximal (n=5)				Early distal (n=5)				Urine (n=10)			
	Control	Moxonidine	Difference	P	Control	Moxonidine	Difference	P	Control	Moxonidine	Difference	P
Sodium (%)	70.9±1.1	61.1±1.1	9.8	<0.01	97.1±0.4	94.7±0.2	2.4	<0.01	99.8±0.05	98.6±0.3	1.2	<0.01
Chloride (%)	54.3±1.8	44.5±1.8	9.8	<0.01	95.8±0.5	93.2±0.3	2.6	<0.01	99.5±0.1	95.9±0.6	3.6	<0.01
Potassium (%)	64.4±1.8	55.4±1.3	9.0	<0.01	95.1±1.2	95.1±1.4	0	n.s.	91.8±1.5	66.1±2.5	25.7	<0.01

induces a marked diuresis and saluresis with a concomitant increase in whole kidney and superficial nephron glomerular filtration rate when applied intravenously to anaesthetized rats.

The free flow micropuncture experiments revealed a clear-cut inhibitory effect of moxonidine on fractional fluid and solute reabsorption in the superficial proximal tubule. Regarding fluid and sodium reabsorption, the proximal effect of moxonidine was progressively diminuted by an increased reabsorption in the loop of Henle and the subsequent nephron segments, leading to an only moderate increase in the fractional excretion rates in urine. Since the loop of Henle and the distal tubules have the ability to adjust their reabsorptive rate to the load delivered into them (Giebisch and Windhager 1973), such compensatory phenomena are regularly observed after diuretic agents acting in the proximal tubule (for a review, see Jackson 1995).

The inhibitory effect of moxonidine on fractional proximal potassium reabsorption was completely compensated in the loop of Henle, but the drug must have induced a net secretion of potassium into the segments lying beyond the early distal tubule because considerable amounts of potassium were excreted in urine. This finding does not necessarily mean that moxonidine affects distal potassium secretion by a direct effect on the tubular epithelium. This effect most probably results from the increased tubular fluid volume and the enhanced sodium load delivered to the distal tubules. These factors are known to promote distal potassium secretion (Wright and Giebisch 1985).

The finding that the fluid volume escaping the proximal tubule is enhanced after moxonidine application may be explained by the increased nephron glomerular filtration rate and/or by a direct action of this drug on the proximal tubule epithelium. The latter assumption is supported by two previously published studies on the effect of imidazoline derivatives on the activity of the proximal Na^+/H^+-exchanger. Schlatter et al. (1997) performed studies on cultured LLC-PK_1 cells, which have properties similar to the proximal tubule. Moxonidine reduced the Na^+/H^+-exchange activity of these cells by 43% when the exchanger was activated by cellular acidification. The effect of moxonidine could be antagonized by the mixed imidazoline receptor/α_2-adrenoceptor antagonist idazoxan, but not by the pure α_2-adrenoceptor antagonist yohimbine, indicating that it was mediated by an activation of imidazoline receptors.

In the study of Bidet et al. (1990) on isolated cells from rabbit renal proximal tubule, a series of imidazoline derivatives were tested which all inhibited the Na^+/H^+-exchanger with an order of potency similar to that previously found for inhibition of [^{3}H]idazoxan binding. The inhibition of the Na^+/H^+-exchanger by this compound did not appear to be related to interaction with α-adrenoceptors since it was observed in the presence of saturating concentrations of the α_2-adrenoceptor antagonist rauwolscine or the α_1-adrenoceptor antagonist prazosine. Since Na^+/H^+-exchange is responsible for a substantial part of total proximal sodium reabsorption, and hence also

of fluid reabsorption, inhibition of this exchanger by moxonidine may well be related to the decreased fluid and solute reabsorption, found after moxonidine in our study.

Schlatter et al. (1997) have also reported that moxonidine inhibited Na^+/H^+-exchange activity in cortical collecting duct segments, which were freshly isolated from rat kidney. In the collecting duct, this transporter resides in the basolateral membrane and its activity is low at resting conditions. It seems, therefore, questionable whether inhibition of this transporter by moxonidine substantially contributes to the diuretic action. In our study, the effect of moxonidine on fractional fluid and sodium excretion was even less pronounced in urine than at the early distal tubular site (see Fig. 1), indicating a compensatory increase, and not a decrease, in tubule fluid reabsorption in the nephron segments lying beyond the early distal tubule, which also include the cortical collecting ducts. Nevertheless, the changes in renal hemodynamics, induced by moxonidine, may have contributed to the diuretic effect. As it was found in our previous study (Hohage et al. 1997), the increase in whole kidney GFR is accompanied by a marked, albeit short-lasting, increase in renal plasma flow. Assuming that medullary blood flow is also enhanced after moxonidine, this effect would lead to a washout of electrolytes from the renal medulla, which would promote diuresis and saluresis.

It has also to be taken into consideration that moxonidine at the dose used in this study reduces the sympathetic activity, regardless of the receptor system involved. The tubular system as most structures in the kidney is densely innervated by the symphathetic nervous system (Muller and Barajas 1972). Thus, it is possible that inhibition of the sympathetic drive to the kidney by moxonidine is related to the effects observed. Furthermore, independently from decreased central sympathetic outflow, moxonidine has also been found to directly inhibit noradrenaline release in the rat kidney (Bohmann et al. 1994).

Taken together, the results of this study have shown that moxonidine induces diuretic and saluretic effects in anaesthetized rats. This effect may be ascribed to an increase of GFR and renal plasma flow, and to a decreased fractional fluid and solute reabsorption in the proximal tubule. The latter effect may be related to an inhibition of the proximal Na^+/H^+-exchanger.

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