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# Visualization of serotonin effects on renal vessels of rats

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**Visualization of serotonin effects on renal vessels of rats.** We studied the effects of serotonin (5-hydroxytryptamine, 5-HT) on glomerular blood flow (GBF) and on renal vessel diameters in the hydronephrotic kidney and in vascular casts of normal kidneys of rats. 5-HT (60 min after local application of $10^{-8}$ mol · liter$^{-1}$) constricted the arcuate arteries (−10 ± 2% to −14 ± 2%, mean ± SEM), dilated the interlobular arteries (+13 ± 2%) and afferent arterioles (+17 ± 3%), and decreased GBF (−44 ± 5%). In contrast to normal autoregulation, reduction of renal perfusion pressure after local application of 5-HT from 118 ± 3 mm Hg by 10 and 20 mm Hg reduced GBF by 12 ± 2% and 23 ± 3%, respectively. The 5-$HT_2$ antagonist, ritanserin (60 min after local application of $10^{-6}$ mol · liter$^{-1}$), dilated all preglomerular vessels and increased GBF. In the presence of ritanserin, 5-HT lost nearly all vascular effects. During infusion of 5-HT (5 μg · min$^{-1}$ i.v. for 20 min) vascular reactions were similar to those under local application. After cyclooxygenase inhibition with indomethacin, infusion of 5-HT failed to constrict the arcuate arteries whereas vasodilation of the small preglomerular vessels remained unaffected. Analyzing vascular casts of normal kidneys we observed considerable vascular spasms and an average vasoconstriction of the interlobar arteries of 19 ± 9% after i.v. infusion of 5-HT. We believe that 5-HT decreases GBF by 5-$HT_2$ receptor-mediated constriction of the large renal vessels which are modulated by the prostaglandin system, whereas 5-HT dilates the small preglomerular vessels, most likely via 5-$HT_1$-like receptors. Furthermore, our data indicate that 5-HT impairs the myogenic component of renal autoregulation in the low pressure range.

Serotonin (5-hydroxytryptamine, 5-HT) belongs to the autacoids functioning as a neurotransmitter and is present in the central nervous system as well as in the periphery [1–4]. During the last decade to the present 5-HT agonists and antagonists have been used in the therapy of hypertension [5–8], and the physiological role of 5-HT in circulation control is of considerable interest but essentially unresolved. Evidence for vasodilation and vasoconstriction of renal vessels after pharmacological doses of 5-HT is provided by several authors [9–14] in agreement with receptor studies in other organs. Generally, 5-$HT_2$ receptors act preferentially by stimulating vasoconstriction of large arterioles, whereas vasodilation is mediated by 5-$HT_1$-like receptors (subgroups of receptors as well as 5-$HT_3$ receptors are described) [4, 15–17]. Recently, the interaction of prostaglandins and 5-HT has been investigated in the canine kidney [10]. This study suggests that the vasodilator effects of 5-HT are sensitive to indomethacin and methysergide, a 5-$HT_1$-like and 5-$HT_2$ receptor antagonist, whereas the dominant constrictor mechanism, evident only after prostaglandin synthetase inhibition, is reversed by ketanserin, a 5-$HT_2$ receptor antagonist. An interaction between 5-HT and prostaglandins has also been reported for the isolated perfused rat kidney [18]. In the intact rat kidney, however, 5-HT was found to act independently of the prostaglandin system [19]. Furthermore, by means of angiography vasoconstriction mediated by 5-$HT_2$ receptors was observed at the conduit renal arteries of the rabbit [12] and dog [13].

Since we developed a technique to visualize the renal vascular system of rats *in vivo* [20, 21], we studied the effect of 5-HT on different vascular segments of the hydronephrotic kidney using the 5-$HT_2$ receptor antagonist, ritanserin, as well as the cyclooxygenase inhibitor, indomethacin. Our primary results were: vasoconstriction of the arcuate arteries, vasodilation of the small preglomerular vessels, and a reduction of glomerular blood flow (GBF) during 5-HT application. These effects were almost completely abolished by application of ritanserin which opened up all preglomerular vessels. Cyclooxygenase inhibition by indomethacin reduced the luminal diameter of all vessels of the hydronephrotic kidney and decreased GBF. Under this condition, 5-HT did not induce further vasoconstriction of the arcuate arteries, whereas the ability to dilate the small preglomerular vessels remained unchanged. Furthermore, GBF did not increase significantly. Utilizing the vascular casting technique, we observed vasoconstriction and locally confined spasms of the interlobar arteries during 5-HT application in the normal rat kidney.

## Methods

### *Induction of hydronephrosis*

In this study 26 female Wistar rats (body weight ranging from 170 to 260 g) were used. Preparation and experimental procedures have previously been described in detail [20]. Briefly, the rats were anesthetized with pentobarbital (40 mg · kg$^{-1}$ i.p., Nembutal$^R$, Ceva, Bad Segeberg, Germany) and a permanent ligation on the left ureter was carried out. In the next eight to twelve weeks, a unilateral hydronephrosis developed.

### *Preparation of the hydronephrotic kidney*

Rats were anesthetized with thiobutalbarbital (100 mg · kg$^{-1}$ i.p., Inactin$^R$, BYK Gulden, Konstanz, Germany). A rectal temperature probe and a heating table were used to maintain the

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body temperature at 37°C. The trachea was intubated and the left jugular vein was cannulated for continuous infusion of isotonic saline (3 ml · $hr^{-1}$) and for infusion of drugs. A catheter was inserted into the left femoral artery for measuring systemic blood pressure continuously.

The kidney was exposed by a left subcostal flank incision and split carefully along the greater curvature with a thermal cautery. The ventral half of the kidney was sutured to a semicircular-shaped wire frame. Blood flow and innervation remained intact after this preparation. The wire frame with the fixed and spread kidney was placed in a Plexiglas chamber suitable for *in vivo* transillumination microscopy. The entry of the renal hilus into the chamber was sealed with silicon grease. The chamber was filled with 50 ml of an isotonic, isocolloidal solution (Haemaccel$^{R}$, Behring, Marburg, Germany). By means of a feedback control system, the tissue bath was kept at a constant temperature of 37°C. After the preparation, each kidney was allowed to adapt to the tissue bath conditions for one hour before the experiments were begun.

In some experiments, a ligature was tied around the abdominal aorta above the renal arteries to lower renal perfusion pressure. The ligature could be tightened gradually with a microscrew for controlled reduction of downstream pressure.

### *Microscopy*

The split hydronephrotic kidney was visualized with a Leitz Ultropac objective UO-55 (water immersion). The microscopical image was recorded with a television camera and a videotape recorder on a calibrated monitor. The luminal vessel diameters were measured directly from the monitor, and the *in vivo* diameters were calculated using the magnification parameters (linear magnification about 3000-fold). Diameter changes of 1 $\mu$m or less could be measured with this magnification. To assess renal blood flow in each kidney, a dye bolus (3% lissamine green) was injected into the jugular vein. A dye arrival time at a selected glomerulus of 2.5 seconds or less was considered to represent an adequate tissue perfusion, based on a similar procedure carried out on normal rat kidneys.

### *Renal vascular segments*

Measurements of the following vessel segments were carried out, the vessels being identified according to the branching pattern of the vessels from the selected cortical glomerulus: (1) proximal arcuate artery (near the interlobar artery), (2) distal arcuate artery (near the interlobular artery), (3) proximal interlobular artery (near the arcuate artery), (4) distal interlobular artery (near the afferent arteriole), (5) proximal cortical afferent arteriole (near the interlobular artery), (6) distal cortical afferent arteriole (at the narrowest segment before entering the glomerulus), (7) proximal cortical efferent arteriole (within 50 $\mu$m after leaving the glomerulus), (8) distal cortical efferent arteriole (near the welling point).

### *Glomerular blood flow*

GBF was determined in the efferent arteriole by a red blood cell velocity tracking correlator (Model 102B, IPM, San Diego, California, USA) [22]. Two photodiodes obtained photometric signals from the moving blood cells. The average time delay between similar events in upstream and downstream signals indicates the transit time the cells need to traverse the interdiode space. GBF was calculated from the measured red cell velocity, from the vessel diameter assuming a circular cross section, and from a constant factor of 0.77 correcting for the Fahraeus effect [23].

### *Experimental protocol*

In the following, specified concentrations for locally applied drugs refer to the final concentration of the drug in the tissue bath. Every protocol started with a control period. Each protocol was made up of several periods during which systemic blood pressure, GBF, and vascular diameters were measured. At the end of each protocol $10^{-6}$ mol · $liter^{-1}$ acetylcholine (Serva, Heidelberg, Germany) and $10^{-5}$ mol · $liter^{-1}$ nitroprusside (Merck, Darmstadt, Germany) were locally applied to confirm vascular reactivity.

In the first series ($N$ = 7), $10^{-8}$ mol · $liter^{-1}$ 5-HT (5-HT creatinine sulfate, Serva) were added to the tissue bath. Measurements were performed after 10, 20, 30, 45, and 60 minutes. Seventy minutes after the local application of 5-HT, renal perfusion pressure was reduced by two to three steps. Each step in which renal perfusion pressure was reduced by 10 mm Hg lasted for about six minutes. Measurements were performed in a stable state five minutes after the pressure reduction.

In the second series ($N$ = 8) $10^{-6}$ mol · $liter^{-1}$ of the 5-$HT_2$ receptor antagonist ritanserin (Janssen, Beerse, Belgium) were locally applied. Measurements were performed after 10, 20, 30, 45, and 60 minutes.

The third series of experiments ($N$ = 5) was done to test the effects of 5-HT after 5-$HT_2$ receptor blockade. To this end, $10^{-6}$ mol · $liter^{-1}$ ritanserin were given first and after 60 minutes $10^{-8}$ mol · $liter^{-1}$ 5-HT were added to the tissue bath. Measurements were performed 30 and 60 minutes after each application.

In the fourth series ($N$ = 5) the effects of intravenously infused 5-HT and a possible interaction of 5-HT with the prostaglandin system were studied. First, 5-HT was intravenously infused for 20 minutes at a constant rate of 5 $\mu$g · $min^{-1}$ ($1.2 \cdot 10^{-8}$ mol · $min^{-1}$) dissolved in 37.5 $\mu$l · $min^{-1}$. Measurements were done 10 and 20 minutes after the beginning of the infusion. The next measurement was performed 30 minutes after the infusion stop. Forty minutes after the infusion stop, 1 mg · $kg^{-1}$ of the cyclooxygenase inhibitor indomethacin (Sigma, Deisenhofen, Germany) was intravenously injected as a bolus followed by a continuous infusion for 60 minutes of 1/60 mg · $kg^{-1}$ · $min^{-1}$ indomethacin dissolved in 25 $\mu$l · $min^{-1}$. Measurements were performed 10 and 60 minutes after the start of the infusion. Immediately after the indomethacin infusion a second infusion of 5-HT was started with the identical protocol as the first one.

### *Preparation of casts*

To study the effects of 5-HT on the interlobar arteries in the normal kidney, casts of the kidneys of twelve female Wistar rats weighing 180 to 260 g were prepared. The animals were anesthetized with 100 mg · $kg^{-1}$ thiobutalbarbital i.p., tracheotomized, and the left jugular vein was cannulated. The abdominal aorta was exposed by a median abdominal incision and a catheter was inserted in the aorta caudal to the renal arteries in a retrograde direction for measuring systemic blood pressure continuously and for the application of various perfusates.

In six rats, 5-HT was intravenously infused for five minutes at

**Table 1.** Control values of diameters, GBF, blood pressure and body weight (mean ± SEM)

| | Series 1 ($N$ = 7) | Series 2 ($N$ = 8) | Series 3 ($N$ = 5) | Series 4 ($N$ = 6) |
|---|---|---|---|---|
| Arcuate artery proximal *μm* | 53.5 ± 3.5 | 67.4 ± 4.5 | 69.6 ± 5.6 | 71.5 ± 3.2 |
| Arcuate artery distal *μm* | 37.6 ± 3.3 | 41.9 ± 1.5 | 47.5 ± 3.4 | 45.7 ± 4.0 |
| Interlobular artery proximal *μm* | 23.6 ± 0.9 | 26.9 ± 1.0 | 26.5 ± 1.9 | 27.6 ± 1.1 |
| Interlobular artery distal *μm* | 12.8 ± 0.6 | 14.0 ± 0.9 | 12.8 ± 0.4 | 13.6 ± 1.2 |
| Afferent arteriole proximal *μm* | 8.4 ± 0.5 | 10.4 ± 0.9 | 9.7 ± 0.4 | 10.6 ± 0.4 |
| Afferent arteriole distal *μm* | 7.2 ± 0.5 | 8.3 ± 0.7 | 7.9 ± 0.4 | 8.7 ± 0.9 |
| Efferent arteriole proximal *μm* | 10.2 ± 0.5 | 10.7 ± 1.4 | 10.8 ± 1.1 | 11.0 ± 0.8 |
| Efferent arteriole distal *μm* | 15.8 ± 1.1 | 17.4 ± 1.0 | 14.6 ± 1.0 | 15.5 ± 1.3 |
| GBF $nl \cdot min^{-1}$ | 40.2 ± 6.1 | 46.7 ± 5.9 | 53.0 ± 7.5 | 63.3 ± 11.0 |
| Blood pressure *mm Hg* | 107.9 ± 3.1 | 108.8 ± 3.9 | 105.0 ± 4.5 | 105.8 ± 2.4 |
| Body weight *g* | 204.3 ± 6.1 | 230.6 ± 5.9 | 224.0 ± 15.2 | 215.0 ± 2.2 |

a rate of 4 $\mu g \cdot min^{-1}$ and in one rat at a rate of 10 $\mu g \cdot min^{-1}$. As controls, five rats were infused with isotonic saline only (50 $\mu l \cdot min^{-1}$ for 5 min). Prior to the infusion period all rats received 1.5 ml (7500 IU) heparin (Braun Melsungen, Melsungen, Germany). Immediately after the infusion period the vena cava was cut open, the aorta was clamped above the renal arteries, and pressure-controlled perfusion fixation was begun through the aortic catheter with 20 ml 1.5% glutaraldehyde in 0.1 M phosphate and 0.1% cacodylate buffer at a temperature of 37°C. Thereafter, Batson's no. 17 casting resin (Polysciences, Warrington, Pennsylvania, USA) was injected through the aortic catheter. The resin was prepared using the proportions of 4:1:0.1 for monomer, catalyst, and promoter, respectively, with red pigment added. After the resin had hardened, the left kidney was removed and placed in 40% potassium hydroxide for digestion of the tissue. The casts were then rinsed thoroughly with distilled water and examined by light microscopy.

### *Vessel morphometry*

The microscopical image of the casts was recorded with a video camera, digitized, and interactively measured with a digital image processing board (MVP-AT, Matrox, Dorval, Quebec, Canada). The interlobar arteries were identified according to their branching pattern. Two to four arteries per cast were selected at random for the measurement. Each interlobar artery was divided into several segments along which the vascular diameters remained fairly constant. For each segment the diameter and the length were determined. To quantify the vasoconstriction induced by 5-HT, we calculated an average diameter for each vessel by weighting each segmental diameter with the segment length. Only vessels longer than 7 mm and their segments up to a distance of 7 mm from the origin of the artery were used to suppress the dependence of the average diameter from the individual vessel length. The average vessel diameters in each animal were combined to a mean diameter per animal.

### *Statistics*

Results are given as mean ± SEM. Changes of vascular diameters and GBF are given as percent changes from the control value. The statistical significance of multiple interventions was calculated by analysis of variance and a post-hoc $t$-test. The mean diameters of the interlobar arteries per animal measured in the kidney casts were tested for statistically

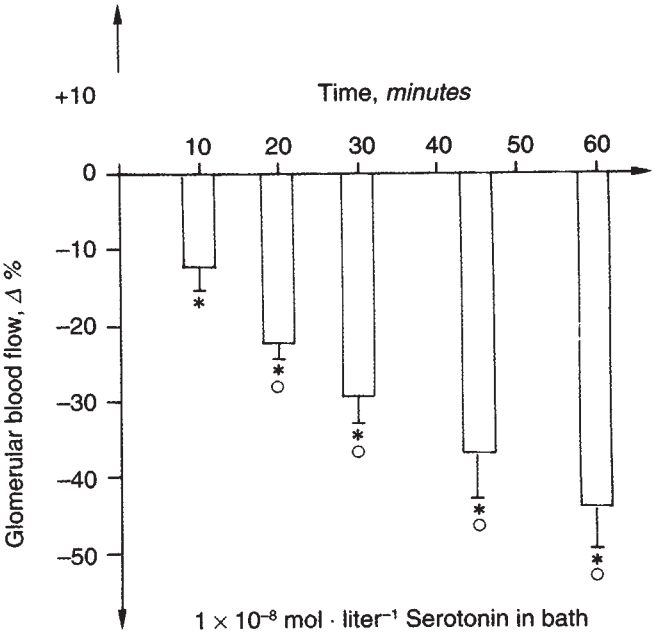


**Fig. 1.** *Percent changes of GBF versus time after local application of $10^{-8}$ mol · liter$^{-1}$ 5-HT (series 1).* $N$ = 7; data are mean ± SEM. * $P <$ 0.05 vs. control; ° $P < 0.05$ vs. previous value.

significant difference by use of the non-parametric permutation-test of Pitman. Values of $P < 0.05$ were considered to be significant.

## Results

The control values of the vessel diameters, GBF, mean arterial pressure, and body weight are summarized in Table 1.

### *Series 1*

In the first series of experiments ($N$ = 7) 5-HT caused a time-dependent decrease of GBF (Fig. 1). Ten minutes after local application of $10^{-8}$ mol · liter$^{-1}$ 5-HT a reduction of GBF by 13 ± 3% was observed, whereas 60 minutes later GBF had decreased to 56 ± 5% of the control value. In some additional experiments we continued the measurements up to 120 minutes after local 5-HT application without observing further changes.

Parallel to the changes of GBF the luminal diameters also changed time-dependently (Fig. 2). Ten minutes after 5-HT

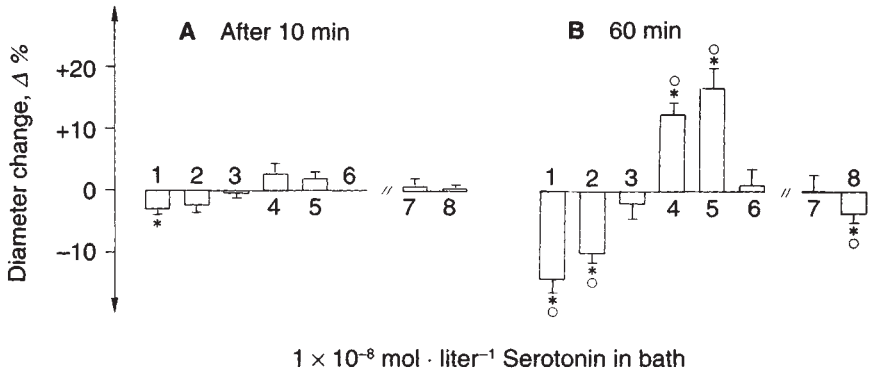


**Fig. 2.** *Percent changes of renal vascular diameters 10 and 60 minutes after local application of $10^{-8}$ mol · liter$^{-1}$ 5-HT (series 1).* $N = 7$; data are mean ± SEM. * $P < 0.05$ vs. control; ° $P < 0.05$ vs. 10 min. Preglomerular: (1) arcuate arteries-proximal; (2) arcuate arteries-distal; (3) interlobular arteries-proximal; (4) interlobular arteries-distal; (5) afferent arterioles-near interlobular arteries; (6) afferent arterioles-near glomeruli. Postglomerular: (7) efferent arterioles-near glomeruli; (8) efferent arterioles-near welling point.

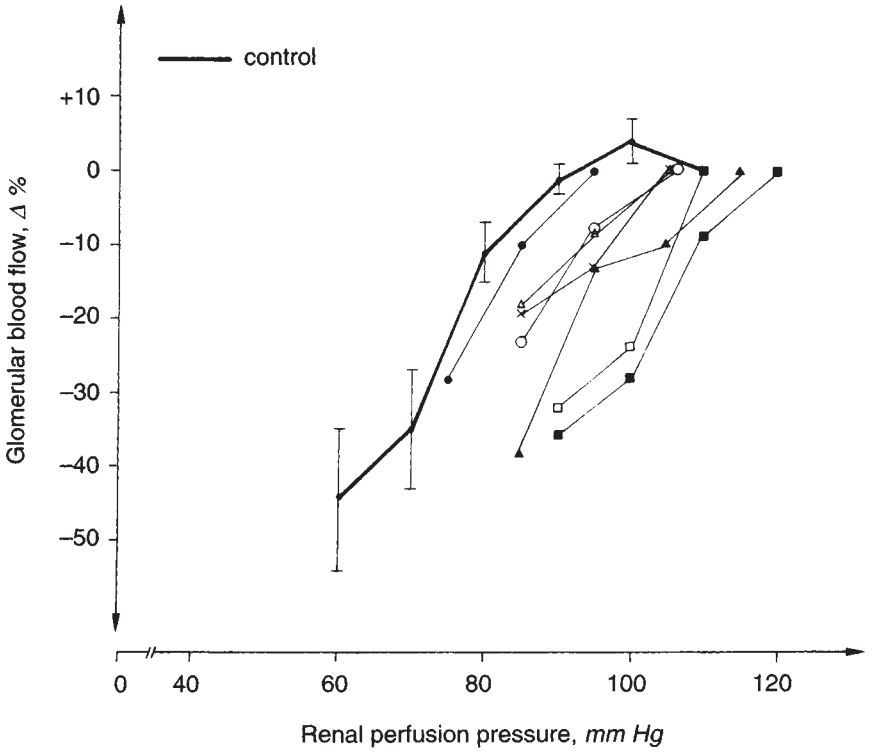


**Fig. 3.** *Percent changes of GBF to reduction of renal perfusion pressure 70 minutes after local application of $10^{-8}$ mol · liter$^{-1}$ 5-HT (series 1).* The curve of each animal is represented by a different symbol and a thin line. The bold line corresponds to control conditions (values are taken from [24]). Note the immediate fall of GBF in response to renal perfusion pressure reduction during 5-HT. $N = 6$; data are mean ± SEM.

application only the proximal arcuate arteries showed a slight luminal diameter reduction. Sixty minutes after local 5-HT application the proximal and distal arcuate arteries had constricted by 14 ± 2 and 10 ± 2%, respectively, whereas the distal interlobular arteries and the afferent arterioles near the interlobular arteries had dilated by the same magnitude (+13 ± 2% and +17 ± 3%, respectively). The efferent arterioles were narrowed to a negligible extent only near the welling points.

Reduction of renal perfusion pressure by clamping the aorta 70 minutes after local application of 5-HT led to a simultaneous reduction of GBF (Fig. 3). In contrast to the usually maintained autoregulation of GBF in the hydronephrotic kidney [24], reduction of renal perfusion pressure from an initial value of 118 ± 3 mm Hg by 10 and 20 mm Hg caused GBF to fall by 12 ± 2% and 23 ± 3%, respectively, in the presence of 5-HT. In accordance with this result renal perfusion pressure reduction induced no autoregulative vasodilation.

### *Series 2*

In the second series of experiments ($N = 8$) local application of $10^{-6}$ mol · liter$^{-1}$ of the 5-HT$_2$ receptor antagonist ritanserin increased GBF also in a time-dependent manner (Fig. 4). In this series nearly all preglomerular vessels dilated significantly already 10 minutes after local ritanserin application (Fig. 5). After 60 minutes the luminal diameters had increased by 8 to 21%. The diameters of the efferent arterioles remained unchanged.

### *Series 3*

In the third series of experiments ($N = 5$) the effect of 5-HT after application of ritanserin (both applied locally) was observed (Fig. 6). Under this condition 5-HT evoked neither dilation nor constriction in any vessel with the exception of the

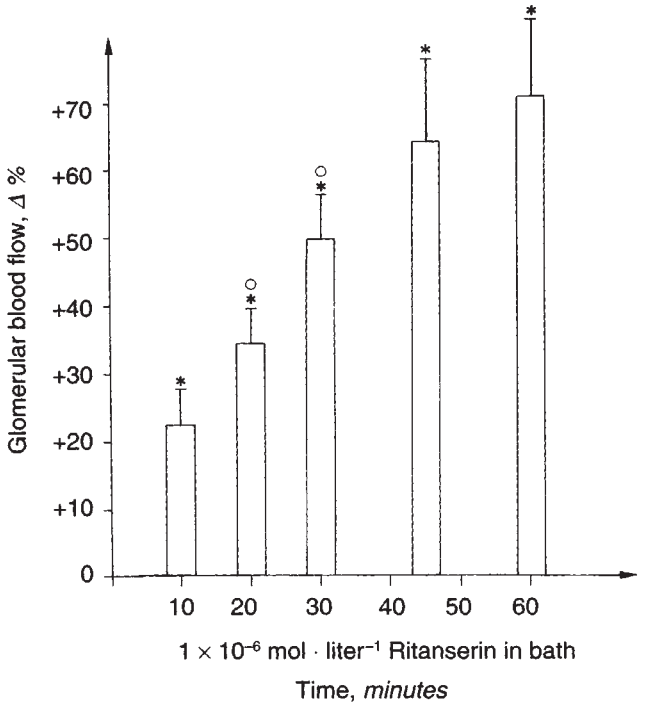


**Fig. 4.** *Percent changes of GBF versus time after local application of $10^{-6}$ mol · liter$^{-1}$ ritanserin (series 2).* $N = 8$; data are mean ± SEM. * $P < 0.05$ vs. control; ° $P < 0.05$ vs. previous value.

proximal arcuate artery which constricted by 4 ± 1%. GBF decreased by 17 ± 5%.

*Series 4*

In the fourth series of experiments ($N = 6$) we tested the effect of 5-HT before and after indomethacin treatment. In this series 5-HT was infused intravenously for 20 minutes each time at a dose of 5 $\mu g \cdot min^{-1}$ dissolved in 37.5 $\mu l \cdot min^{-1}$. As shown in Figure 7A the pattern of renal vascular diameter changes is very similar compared to that 60 minutes after local 5-HT application (cf. Fig. 2). Vasoconstriction of the proximal arcuate arteries (−25 ± 4%) is combined with vasodilation of the distal interlobular arteries (+28 ± 7%) and the afferent arterioles near the interlobular arteries (+24 ± 5%). Likewise, GBF was reduced in a very similar manner compared to local application (cf. Fig. 1).

Thirty minutes after the stop of the first 5-HT infusion GBF and the vessel diameters of the arcuate arteries returned to their initial values, whereas the distal interlobular arteries and the afferent arterioles near the interlobular arteries did not reach their control values (+10 ± 3% and +10 ± 2%, respectively).

Indomethacin induced pre- and postglomerular vasoconstriction in our preparation ranging from −8% to −17%. Accordingly, GBF fell by −35 ± 5%.

Under cyclooxygenase inhibition the second 5-HT infusion failed to constrict the arcuate arteries (Fig. 7B). However, the pattern of vasodilation of the other preglomerular vessels was not affected by the pretreatment with indomethacin. Besides, the diameter of the efferent arterioles near the welling points increased by 15 ± 3%. GBF did not significantly change.

Mean arterial blood pressure (measured continuously in the femoral artery) did not show any significant changes during local application of drugs (series 1 to 3) with the exception of a slight decrease from the initial value of 109 ± 4 mm Hg to 104 ± 4 mm Hg after 60 minutes local application of ritanserin.

In the fourth series of experiments mean arterial blood pressure fell from 106 ± 2 mm Hg under control conditions to 93 ± 3 mm Hg 20 minutes after beginning the first 5-HT infusion, and rose again 30 minutes after the end of the 5-HT infusion to 101 ± 3 mm Hg. Twenty minutes after starting the second 5-HT infusion mean arterial pressure equaled 91 ± 7 mm Hg which did not significantly differ from 103 ± 5 mm Hg, the value at the end of the indomethacin infusion.

*Vascular casts*

Figure 8a shows the cast of a normal rat kidney. 5-HT infused intravenously at a rate of 4 $\mu g \cdot min^{-1}$ for five minutes induced a visible vasoconstriction of the large renal arteries as well as locally confined spasms (Fig. 8b). At a rate of 10 $\mu g \cdot min^{-1}$ (Fig. 8c) spasms occurred along considerable distances reducing the luminal diameter to almost zero. Thus, neither a complete filling nor a proper preparation of the arterial tree was possible.

The vascular casts obtained under control conditions and after five minutes infusion of 5-HT at a rate of 4 $\mu g \cdot min^{-1}$ were quantitatively evaluated. To this end, the mean diameter as well as the maximum and minimum diameter of the interlobar arteries were plotted versus the distance from the origin of the artery (Fig. 9). The step-like changes of the diameters were caused by the quantization error of the measurement, by abrupt changes of the vessel caliber at branching points, and by spasms after 5-HT application. There was a large anatomical variability among the interlobar arteries of the control animals. Nevertheless, the diameter of each interlobar artery generally decreased from an initial value of about 300 $\mu m$ down to about 150 $\mu m$. Furthermore, the effects of 5-HT on the interlobar arteries conspicuously varied within one animal as well as among the animals. The mean length of the interlobar arteries was 9130 ± 510 $\mu m$ in the control group (5 animals, 12 interlobar arteries) and 7870 ± 330 $\mu m$ in the group receiving 5-HT (6 animals, 19 interlobar arteries). The mean diameter (as defined in **Methods**) of the interlobar arteries was 245 ± 16 $\mu m$ in the control group (5 animals, 11 interlobar arteries), whereas it was reduced by 19 ± 9% to 199 ± 17 $\mu m$ after infusion of 5-HT (6 animals, 16 interlobar arteries).

## Discussion

During local as well as intravenous application of 5-HT we observed vasoconstriction of the arcuate arteries, vasodilation of the interlobular arteries and of the afferent arterioles, and a reduction of GBF in the hydronephrotic kidney. For the present, it seems reasonable to assume a similar action of 5-$HT_1$-like and 5-$HT_2$ receptors in the kidney as in other organs which is supported by several studies [9–14]. Therefore, the vasoconstriction of the large renal vessels should be mediated predominantly by 5-$HT_2$ receptors and the vasodilation of the small preglomerular vessels by 5-$HT_1$-like receptors. In addition, a part of the vasodilation may be caused by autoregulation in response to the increased pressure drop along the large arteries. Since the constriction of the large vessels is of the same magnitude compared to the vasodilation of the small

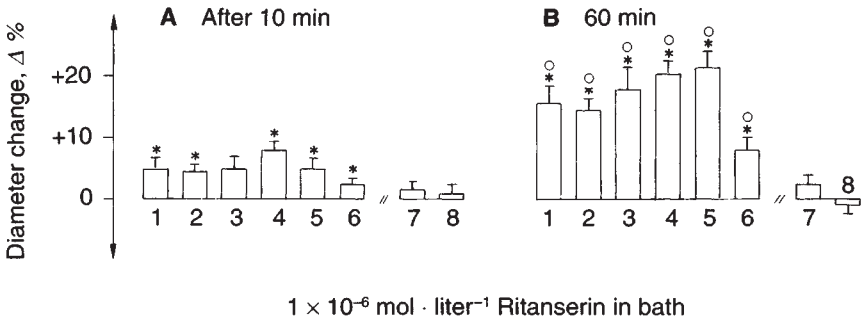


**Fig. 5.** *Percent changes of renal vascular diameters 10 and 60 minutes after local application of $10^{-6}$ mol · liter$^{-1}$ ritanserin (series 2).* $N = 8$; data are mean ± SEM. * $P < 0.05$ vs. control; ° $P < 0.05$ vs. 10 min. Preglomerular: (1) arcuate arteries-proximal; (2) arcuate arteries-distal; (3) interlobular arteries-proximal; (4) interlobular arteries-distal; (5) afferent arterioles-near interlobular arteries; (6) afferent arterioles-near glomeruli. Postglomerular: (7) Efferent arterioles-near glomeruli; (8) efferent arterioles-near welling point.

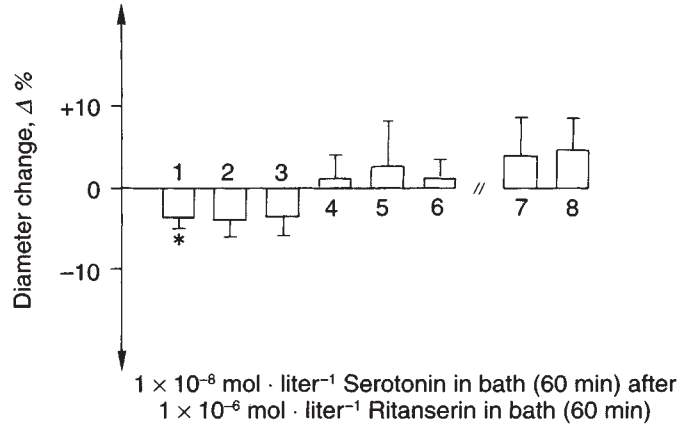


**Fig. 6.** *Percent changes of renal vascular diameters 60 minutes after local application of 5-HT ($10^{-8}$ mol · liter$^{-1}$) given 60 minutes after local application of ritanserin ($10^{-6}$ mol · liter$^{-1}$) versus ritanserin only (series 3).* $N = 5$; data are mean ± SEM. * $P < 0.05$ vs. $1 \times 10^{-6}$ mol · liter$^{-1}$ ritanserin 60 min in bath.

vessels, the vessels upstream to the arcuate arteries which are not routinely accessible by our technique must be constricted during 5-HT application to account for the reduction in GBF. For the normal kidney, we were able to measure such a diameter reduction of the interlobar arteries in the kidney casts. Since in the hydronephrotic kidney the vascular resistance of the large vessels (arcuate and interlobar arteries) is nearly twice that of the small vessels (interlobular arteries and afferent arterioles) [25] the reduction of GBF is in good agreement with the observed vascular reactions.

During intravenous infusion of 5-HT the vascular effects reached a stable state within a few minutes whereas during local application a stable state was reached after one hour. This difference may be caused by long diffusion distances to the large vessels relevant to locally applied substances. Furthermore, intravenously applied substances approach the vascular wall from intraluminal sites via the endothelium whereas locally applied substances preferentially act on the vascular wall from extraluminal sites. In the case of intra- and extraluminal application of 5-HT an asymmetry of the contractile response of isolated arteries has indeed been reported [26, 27]. This asymmetry might be caused, at least in part, by the endothelium as there are indications that the endothelium is involved in the mediation of the vascular effects of 5-HT [4, 27, 28]. However, the ability of 5-HT to induce renal vasodilation via endothelial receptors is controversial [14, 29]. In contrast to the effects of 5-HT local application of angiotensin II constricts the small pre- and postglomerular vessels of the hydronephrotic kidney within a few minutes [30].

Ritanserin is known as a selective 5-$HT_2$ receptor antagonist [31]. During local application of ritanserin all preglomerular vessels dilated. Therefore, an endogenous tonus of 5-HT should be present in the hydronephrotic kidney. By that, the dilation of the large arteries is due to the blockade of 5-$HT_2$ receptors. Concerning the small vessels, the interpretation of their dilation is not straightforward. On the one hand, blockade of 5-$HT_2$ receptors could lead to a redistribution of endogenous 5-HT to 5-$HT_1$-like receptors. On the other hand, the small vessels may additionally possess 5-$HT_2$ receptors. This assumption is able to explain the vasodilation of the small vessels by combining blockade of the 5-$HT_2$ receptors and redistribution of 5-HT. During 5-HT application, the vasodilation of the small vessels remains still plausible since the autoregulative and 5-$HT_1$-induced vasodilation should exceed a 5-$HT_2$-mediated constriction.

During ritanserin application 5-HT did not exhibit any vascular effects with the exception of a slight constriction of the proximal arcuate arteries and a moderate reduction of GBF. Therefore, the 5-$HT_2$ receptor-mediated constriction of the large arteries is almost completely blocked by ritanserin. On the other hand the 5-$HT_1$-like receptor-mediated dilation of the small vessels should not be affected by the presence of ritanserin at first sight. However, the inefficacy of 5-HT seems to be plausible for two reasons. First, the small vessels were already dilated up to 20% by the application of ritanserin which reduces the available dilator capacity. Second, the 5-$HT_1$-like receptors could already be completely stimulated due to the redistribution of 5-HT discussed above.

Besides its direct action on vascular smooth muscle, 5-HT is also known to induce vasodilation by 5-$HT_1$-like receptor-mediated inhibition of noradrenaline release from presynaptic terminals of postganglionic sympathetic nerves [4]. $\alpha$-Adrenergic blockade by phentolamine and $\beta$-adrenergic blockade by propranolol have no vascular effects in our hydronephrotic kidney preparation [32]. Therefore, the 5-HT-induced dilation of the small preglomerular vessels does not appear to be caused by inhibition of noradrenaline release. It is further unlikely that 5-HT constricts the large vessels indirectly by an increased release of noradrenaline from sympathetic nerve terminals, since ritanserin, which possesses low *in vitro* and almost no *in vivo* $\alpha_1$-affinity [33], is able to block the 5-HT-induced vasoconstriction. Moreover, an unaffected vascular response to 5-HT

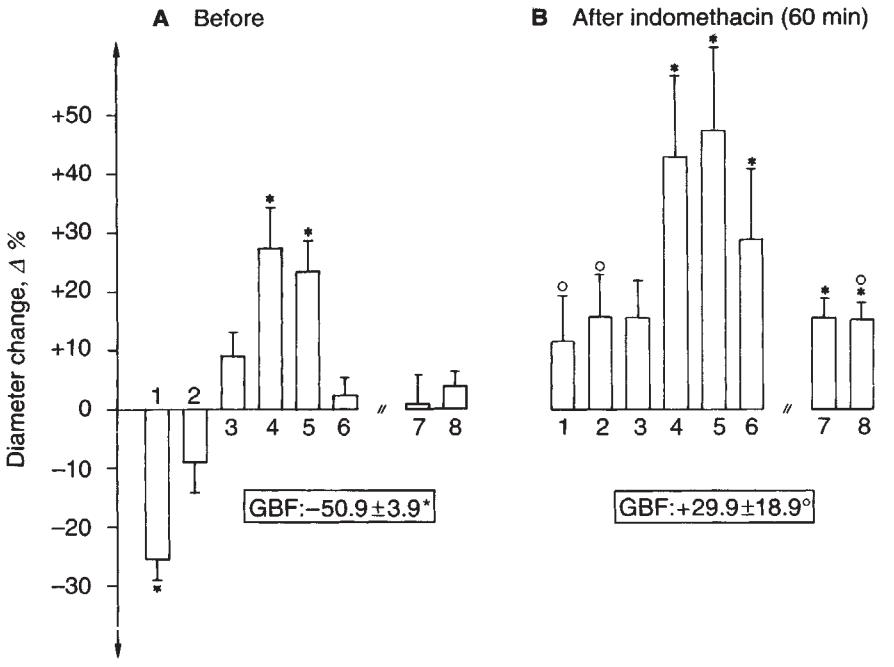


**Fig. 7.** *Percent changes of renal vascular diameters and GBF during intravenous infusion of 5-HT (5 μg · min$^{-1}$ for 20 min) before and after treatment with indomethacin (1 mg · kg$^{-1}$ i.v. for 60 min after bolus injection of 1 mg · kg$^{-1}$ i.v.; series 4).* $N = 6$; data are mean ± SEM. **A.** GBF = −50.9 ± 3.9*. * $P < 0.05$ vs. control. **B.** GBF = +29.9 ± 18.9°. * $P < 0.05$ vs. indomethacin (60 min); ° $P < 0.05$ vs. serotonin before indomethacin.

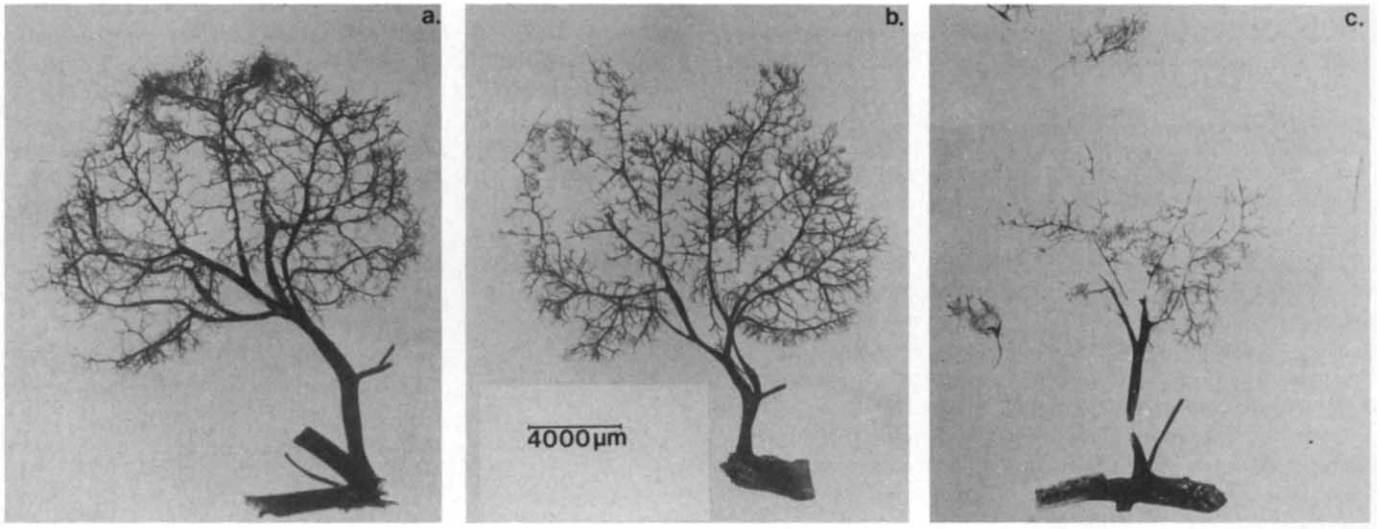


**Fig. 8.** *Vascular casts of the normal rat kidney.* **(a)** control; **(b)** intravenous infusion of 20 μg 5-HT in 5 min; **(c)** intravenous infusion of 50 μg 5-HT in 5 min. Note the incomplete filling and the multiple fragments in (c) which were due to vascular spasms.

after α- as well as β-blockade was demonstrated in the cremaster muscle of the rat [17].

During local application of 5-HT autoregulation was abolished in our kidney preparation. We observed the same loss of autoregulation after vasodilation by calcium antagonists [34]. Apart from a direct action of 5-HT on the contractility of vascular smooth muscle, the responsiveness of the small vessels to autoregulative stimuli could already be exhausted due to the constriction of the large vessels acting as an autoregulative stimulus. By this assumption, renal perfusion pressure reduction would result in an immediate fall of GBF since autoregulation is predominantly mediated by vasodilation of the small vessels [35]. As we only decreased renal perfusion pressure in these experiments we cannot exclude that autoregulation was maintained at higher pressures.

For the rat, 5-HT and direct agonists are known to increase renal vascular resistance in conscious [36, 37] or anesthetized animals [19] as well as in the isolated perfused kidney preparation [9, 11, 18]. Using selective 5-HT$_2$ receptor agonists and antagonists, the increase of renal vascular resistance was shown to be mediated by 5-HT$_2$ receptors [37, 38]. Lameire et al [39] demonstrated that blockade of 5-HT$_2$ receptors by a low dose of Ketanserin increased the PAH clearance and enhanced renal autoregulation in the low pressure range. From this, it could be deduced that 5-HT impairs renal autoregulation in the low pressure range and that the normal kidney is subject to a

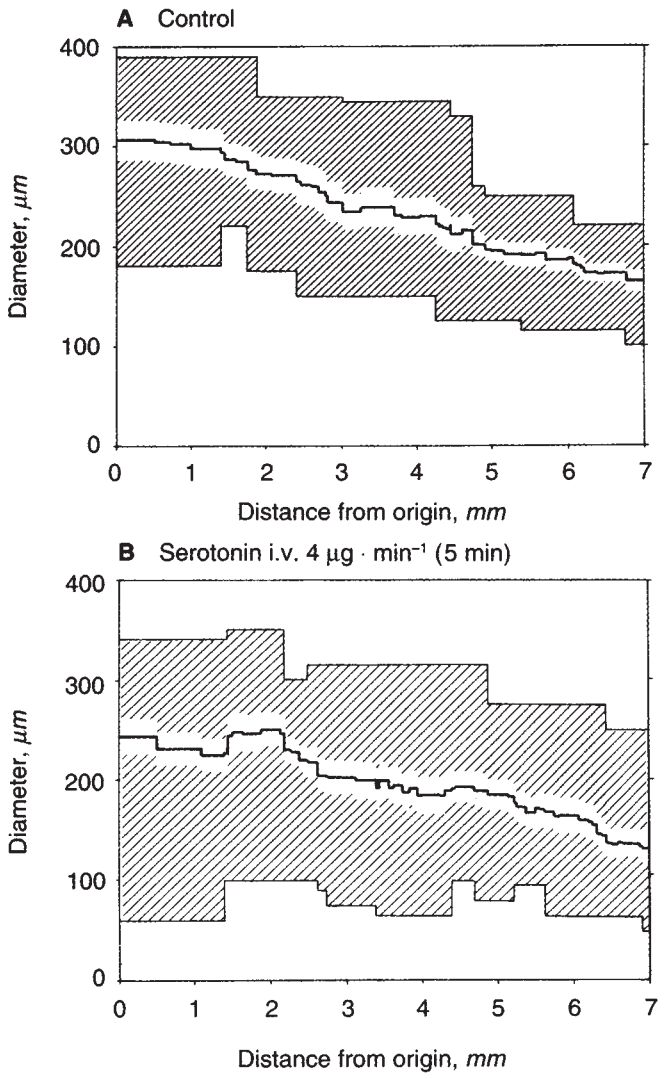


**Fig. 9.** *Morphometry of the interlobar arteries during control (A) and after intravenous infusion of 5-HT (B; 20 μg in 5 min) in the normal rat kidney.* The mean diameter (bold line) of the interlobar arteries as measured in the kidney casts is plotted versus its distance from the origin of the vessels. The range between minimum and maximum vessel diameters is indicated by the hatched area. The blank area along the bold line corresponds to the range of mean ± SEM. After infusion of 5-HT the mean diameter decreased by about 50 μm and the diameter range increased due to vascular spasms. A. Mean diameter 245 ± 16 μm. B. Mean diameter 199 ± 17 μm; $P < 0.05$ vs. controls.

basal tonus of 5-HT. All these findings for the rat kidney are in close agreement with our results.

The 5-$HT_2$ receptor-mediated increase of renal vascular resistance after application of 5-HT was also confirmed in the anesthetized as well as in the conscious rabbit [12, 40]. More recently, however, contraction of the isolated renal artery of the rabbit was found to be mediated by 5-$HT_1$-like receptors [41]. In anesthetized dogs intrarenal infusion of 5-HT increased renal blood flow after an initial transient decrease [10, 42, 43], whereas intravenously infused 5-HT caused renal blood flow to fall in conscious dogs [44]. Moreover, Blackshear, Orlandi and Hollenberg [10] concluded from their experiments that in the canine kidney vasoconstriction is mediated by 5-$HT_2$ receptors and vasodilation by 5-$HT_1$-like receptors, whereas the opposite action of 5-HT receptors was inferred by Shoji et al [42].

Further, we studied the effect of cyclooxygenase inhibition on the action of 5-HT. After cyclooxygenase inhibition 5-HT failed to constrict the large vessels and to reduce GBF. Nevertheless, the small vessels were dilated by 5-HT again. Comparing the effects of 5-HT before and after cyclooxygenase inhibition the significant differences are located at the arcuate arteries and at the efferent arterioles near the welling points. Diameter changes of the efferent arterioles at the welling points, which lack vascular smooth muscle cells at this segment, are of minor relevance as they reflect only passive mechanisms. Whereas, for the arcuate arteries, cyclooxygenase inhibition obviously interferes with the 5-$HT_2$ receptor-mediated vasoconstriction. Thus, the 5-$HT_2$ receptor-mediated vasoconstriction is dependent on an intact prostaglandin system. The dilation of the small vessels is not affected by cyclooxygenase inhibition. This dilation appears to be solely caused by 5-$HT_1$-like receptors, since an autoregulative stimulus is scarcely present and the 5-$HT_2$ receptor-mediated constriction is abolished by indomethacin.

Our finding that the prostaglandin system is involved in the vasoconstrictive action of 5-HT is in line with similar findings for the dog [10, 13], the isolated perfused rat kidney [18], and the isolated basilar artery of the rabbit [28]. Despite of this agreement, the vasoconstriction elicited by 5-HT in our preparation was almost completely abolished by prostaglandin synthetase inhibition, as it was likewise reduced in the isolated basilar artery of the rabbit [28], while in the other studies [10, 13, 18] vasoconstriction was enhanced in the absence of prostaglandins. For the normal rat kidney, however, the effects of 5-HT were not affected by indomethacin [19]. Whether these conflicting results reflect differences between species or differences between the experimental models employed remains an open question at present.

To study the effects of 5-HT on the interlobar arteries of the normal kidney we utilized the vascular casting technique validated in mesenteric vessels [45] and in the post-ischemic hydronephrotic kidney [46]. After intravenous infusion of 20 μg 5-HT in five minutes we found a diameter reduction of the interlobar arteries by about 20% and spasms. After infusion of 50 μg 5-HT in five minutes the large renal vessels constricted to such a degree that neither a complete filling nor a proper preparation was possible. These results indicate that the 5-$HT_2$ receptor-mediated constriction of the large vessels observed in the hydronephrotic kidney is also present in the normal kidney.

Arteriograms of the rabbit and the dog revealed the same vascular effects of 5-HT at the renal artery and the conduit renal arteries [12, 13]. The vasoconstrictive effect of 5-HT at the large renal vessels was shown to be mediated by 5-$HT_2$ receptors and to be modulated by prostaglandins [13], supporting our results in the hydronephrotic kidney.

Furthermore, by the quantitative analysis of the vascular casts we are able to provide rough estimates concerning the vascular resistance of the interlobar arteries during control conditions and during application of 5-HT in the normal rat kidney. If we assume the interlobar artery to be a circular shaped tube with linearly decreasing diameter and flow from the beginning to the end, we could calculate the pressure drop along

this vessel by integrating the infinitesimal pressure drops over the total vessel length. The tube resistance and the pressure drop are derived from the law of Hagen-Poiseuille and Ohm's law, respectively. Assuming a renal blood flow of 7 ml · $min^{-1}$ and about 10 interlobar arteries which give rise to about 100 arcuate arteries, the flow at the beginning of the interlobar artery is set to 0.7 ml · $min^{-1}$ and to 0.07 ml · $min^{-1}$ at the end. According to our measurements the diameter is about 300 $\mu$m at the beginning decreasing to about 150 $\mu$m at the end along a distance of about 8 mm (cf. Fig. 9). Since the macroscopic viscosity of blood is about 4 centiPoise the viscosity in that diameter range is set to 3 centiPoise in consideration of the Fahraeus-Lindqvist effect. Employing these assumptions yields the remarkable pressure drop of 18 mm Hg along the interlobar artery. Vasoconstriction of about 20% measured during infusion of 5-HT doubles the resistance of the interlobar artery. These are only approximate values which tend to underestimate the effects of 5-HT as the mean diameters were obtained by averaging over spasms. Nevertheless, these data indicate that the interlobar arteries may substantially contribute to the total vascular resistance of the rat kidney. Thereby, the large renal vessels should play an important role for the regulation of renal blood flow during 5-HT application.

In conclusion, our data indicate that 5-HT decreases renal blood flow by $5\text{-HT}_2$ receptor-mediated vasoconstriction of the interlobar and arcuate arteries. This effect is modulated by the prostaglandin system. The interlobular arteries and afferent arterioles are vasodilated by 5-HT most probably via $5\text{-HT}_1$-like receptors. Furthermore, 5-HT impairs the myogenic component of renal autoregulation in the low pressure range.

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