Beneficial Influence of Ketanserin on Autoregulation of Blood Flow in Post-Ischemic Kidneys

Marleen Verbeke, Bernd Smöllich, Johan Van De Voorde, Leo de Ridder, and Norbert Lameire

The influence of ketanserin, a S₂-serotonergic receptor blocker, on the impaired renal hemodynamics in a clamp model of renal ischemia in rats was investigated in this study. Serotonin-induced vasoconstriction of the renal vascular bed was augmented after ischemia. This constriction is blocked by ketanserin (0.05 mg/kg iv bolus, followed by 0.1 mg/kg per h infusion). The influence of the same ketanserin treatment on the response of RBF versus a stepwise lowering of the renal perfusion pressure was studied in post-ischemic kidneys with an established loss of autoregulation of RBF. An almost perfect restoration of the autoregulatory response was apparent after the S₂-serotonergic antagonism. Despite this beneficial effect on renal hemodynamics, renal function, judged by measurement of GFR and urinary sodium excretion rate, was not influenced by an acute infusion of ketanserin in post-ischemic kidneys. It is suggested that serotonin plays a pivotal role in the suppression of autoregulation of RBF by a S₂-serotonergic receptor-mediated vasoconstrictor effect in the post-ischemic kidney. It most likely masks the potential myogenic dilatory response of the smooth muscle cells in renal preglomerular microvasculature. Restoration of the renal autoregulatory capacity by S₂-serotonergic receptor antagonism could be of clinical relevance in human post-ischemic acute renal failure.

Key Words: 5-Hydroxytryptamine, serotonin, vasoconstriction, arcuate arteries, myogenic response

Renal biopsies obtained from patients during the maintenance phase of ischemic acute tubular necrosis show histological evidence of fresh ischemic lesions, suggesting recurrent intermittent insults of renal hypoperfusion (1). Moreover, in the norepinephrine and renal-artery clamp animal model of post-ischemic acute tubular necrosis, subsequent hemorrhagic blood pressure reduction is associated with functional and histological evidence of recurrent renal damage (2,3). Such ongoing ischemic injury has been explained by a loss of autoregulation of RBF so that the kidneys are unable to protect themselves against subsequent falls in renal perfusion pressure (RPP). A loss of autoregulation of RBF has been reported in a number of post-ischemic (4–7) and in at least one model of toxic acute renal failure, induced by cyclosporine (8).

A number of factors playing a possible role in this loss of autoregulation after acute tubular necrosis have been defined. In both the clamp and the norepinephrine model, structural alterations in the renal vasculature were observed (6,9). Moreover, in the norepinephrine model, increased activity of non-α-adrenergic mechanisms (10), endothelial cell injury, and increased renovascular smooth muscle calcium overload (11) have been described.

In a previous study, we showed that ketanserin, a selective serotonin S₂ antagonist, not only increased RBF and GFR in the normal rat kidney, but also preserved the autoregulation of RBF to perfusion pressures of 70 to 75 mm Hg. In the normal rat kidney, autoregulation of RBF is lost below a perfusion pressure of 90 to 95 mm Hg. These data indicate that a basal serotonin activity influences the hemodynamics in normal kidneys (12). In the same study, we found that serotonin infusion induced a pronounced vasoconstriction, dependent on S₂-receptor stimulation, followed by a less pronounced dose-independent vasodilation, the latter probably mediated by S₁-receptor activation. Furthermore, in a recent study, Endlich et al. (13) visualized the intrarenal vascular effects of serotonin. It was found that basal serotonin S₂ activity predominantly constricts the arcuate arteries, whereas serotonin S₁ activity elicits a vasodilation in interlobular and afferent arterioles. Moreover, administration of serotonin impaired the autoregulation of GFR.

These data led us to investigate whether an S₂-receptor blocker, ketanserin, is able to influence the autoregulatory capacity in post-ischemic kidneys. A possible amelioration of the autoregulation after ischemia by serotonin S₂ blockade could be of obvious clinical importance. It was found that the renal vasoconstrictive response to intrarenal serotonin was aug-
mented after ischemia. This vasoconstriction could be blocked by serotonin S₂ antagonism. The most important observation was, however, that serotonin S₂ antagonism led to the reappearance of the autoregulation of RBF in post-ischemic kidneys.

METHODS

Laboratory Animals

The studies were performed in male Wistar rats (Iffa Credo, Brussels, Belgium) of 285 to 320 g body weight. All animal experimentation was conducted in accord with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Twenty-four h before each surgical intervention, the animals were deprived of food, but received tap water ad libitum. They were anesthetized with an ip injection of sodium pentobarbital (60 mg/kg; Nembutal; Ceva, Brussels, Belgium) and placed on a heating table that maintained body temperature at 37°C.

Study of the Autoregulation of Renal Blood Flow in Control Conditions

The femoral artery and vein were cannulated with polyethylene PE50 tubings. Subsequently, an isotonic saline solution was infused continuously into the femoral vein at a rate of 0.0425 mL/min. The femoral arterial pressure was monitored with a pressure transducer (Harvard Apparatus, Millis, MA) attached to a recorder (Harvard Apparatus). Only animals with a basal mean arterial blood pressure of at least 115 mm Hg were studied.

The aorta and right renal artery were exposed via a small abdominal incision. A string was placed around the aorta above both renal arteries. Both ends of the string were passed through a fixed metallic tube. By gradual compression of the aorta, it was possible to lower the RPP by steps of 5 mm Hg. A small blood-flow sensor with a inner diameter ranging between 0.6 to 0.8 mm (Skalar Medical, Deift, The Netherlands) was placed on the right renal artery, allowing RBF monitoring by an electromagnetic square wave flow meter (Skalar Medical) and recorded on a electronic recording module (Harvard Apparatus). Calibration of the flow probe was conducted according to Arendshorst et al. (14). For intrarenal administration of drugs, a catheter was inserted into the right suprarenal artery, according to a technique described by Smits et al. (15) and as used in our previous experiments (12). In the autoregulation studies, RPP, considered to be equal to the femoral arterial blood pressure, was gradually reduced to 70 mm Hg by steps of 5 mm Hg, and both RBF and RPP were continuously recorded. At every level of RPP, RBF was allowed to equilibrate for at least 2 to 3 min.

Clamping of the Renal Artery

After the first autoregulation curve was obtained, unilateral renal ischemia was provoked by clamping the right renal artery for 40 min. Immediately after clamping, and at regular intervals, the macroscopic aspect of the clamped kidney was verified to assure the completeness of the ischemia. After release of the vascular clamp, and after the completeness of the renal reperfusion was visually observed, the femoral artery and vein were closed and the incisions sutured. The rats recovered with free access to food and water.

Study of the Autoregulation of Renal Blood Flow in Post-I ischemic Conditions

The animals were restudied 48 h or 7 days after renal reperfusion. They were reanesthetized, a tracheotomy was performed, and catheters were placed in the intact femoral vein and artery. The vein was used for systemic administration of either isotonic saline or ketanserin (Janssen Pharmaceutica, Beerse, Belgium). The arterial blood pressure was measured in the femoral artery, and again only animals with a systemic blood pressure of at least 115 mm Hg were studied. The abdominal incision was reopened, and the aorta and right renal artery were freed from surrounding tissue and prepared for the study of autoregulation of RBF as described above. After confirmation of the loss of autoregulation of RBF in this second curve, different study protocols followed.

Study Protocols for Post-I ischemic Kidneys with an Established Loss of Autoregulation of Renal Blood Flow

1. Dose-response studies. Bolus injections of serotonin (5-hydroxytryptamine; Sigma Chemical Company, St. Louis, MO) were delivered into the right renal artery while RBF was continuously measured. Increasing doses of serotonin (0.3, 0.5, 0.75, and 1 μg) were injected before and during ketanserin administration (bolus of 0.05 mg/kg, followed by a sustained infusion of 0.1 mg/kg per h, dissolved in isotonic saline at a rate of 0.0425 mL/min; N = 6) or the same amount of saline (N = 6) as described for the autoregulation studies in the normal kidneys (12). Dose-response curves were studied 48 h after reperfusion.

To exclude eventual enhanced effects of intrarenal doses of serotonin because of the decreased basal RBF in the post-ischemic kidneys, the dose-response study was repeated in six additional animals, using doses lowered to a similar extent as the RBF (80% of the former serotonin doses).

To be sure that the dose of ketanserin used in the present experiments on post-ischemic kidneys was devoid of α-adrenergic blocking activity, as demonstrated in our former study on normal kidneys (12), dose-response curves to intra-renal phenylephrine, a pure α-agonist (0.1, 0.3, 0.5 and 0.7 μg; Sigma Chemical Company, St. Louis, MO), were examined before and during ketanserin administration.

2. Clearance studies. Standard clearances were performed, as described previously (12), at 48 h (N = 15) and 7 days (N = 12) of reperfusion, before and during ketanserin administration (see Protocol 1). In brief, the anesthetized animals were iv-infused with saline at a rate of 0.0425 mL/min. A pressure transducer was connected to the carotid artery for continuous measurement of mean arterial pressure. Inulin (3.3%) was then added to the infusion and administered during 1 h before further urine and blood sampling. In the meantime, both urethers were catheterized (PE-10). Urine of the left and right kidney was collected separately during three consecutive periods of 10 min while the iv infusion with inulin continued. In the middle of the first and third urine collection, a blood sample was taken. Three clearance collections were made before and during the ketanserin administration. Inulin was measured in urine and serum samples with the anthrone method (16); sodium was determined by means of a Klina flame photometer (Beckman Instruments, Fullerton, CA).

3. Autoregulation. Ketanserin: This protocol involved two subgroups of animals. A first group of 15 rats was studied 48
h after renal reperfusion. Ketanserin was applied as described in Protocol 1. In these circumstances, renal vasoconstrictive response to increasing doses of serotonin is blocked, although the vasodilatory response is well preserved (12; present results). After 45 to 60 min of the ketanserin infusion, a third autoregulation curve was obtained while the infusion was continued. In the second subgroup of six animals, the same protocol was performed 7 days after kidney reperfusion.

Saline: A group of animals (N = 10) was studied as described for the ketanserin protocol, except that an isotonic saline solution at a rate of 0.0425 mL/min was administered before and during the third autoregulation analysis. Six animals were studied after 48 h and four animals after 7 days of kidney reperfusion.

Morphological Study

After the last autoregulation curve, the kidneys of six rats at 48 h of reperfusion were perfusion-fixed at a constant pressure of 120 mm Hg by retrograde aortic perfusion with 1.25% glutaraldehyde in 0.1 M cacodylate buffer (17). The kidneys were perfused until a complete washout of the vascular tree was observed, and processed for light microscopy and electron microscopy.

Kidney slices were further immersion-fixed in buffered formalin and embedded in paraffin. Five-μm sections were stained with hemalum-eosin and studied with a Jenaval light microscope (Jena, Germany).

Thick cortical fragments were further immersion-fixed in 2% glutaraldehyde in the same buffer, postfixed in OsO4, and embedded in ERL resin (Serva, Heidelberg, Germany). After staining with uranyl and lead, ultrathin sections were examined by means of a Jeol 1200 EX2 electron microscope (Tokyo, Japan).

Statistical Analysis

The results are given as the means ± SE. Comparison between absolute RBF data were analyzed by the paired or unpaired Wilcoxon's test. Analysis of the autoregulation curves was performed by linear regression analysis of the RPP to RBF data. Differences in results in each protocol were analyzed by ANOVA of the mean slope of the curves was performed by linear regression analysis of the unpaired Wilcoxon's test. Analysis of the autoregulation between absolute RBF data were analyzed by the paired or unpaired Wilcoxon's test. Analysis of the autoregulation curves was performed by linear regression analysis of the unpaired Wilcoxon's test. Analysis of the autoregulation curves was performed by linear regression analysis of the unpaired Wilcoxon's test. Analysis of the autoregulation curves was performed by linear regression analysis of the unpaired Wilcoxon's test.

Table 1 depicts the percentage changes in RBF in response to increasing doses of intrarenal serotonin in both control and ischemic kidneys. 48 h after renal reperfusion. The mean absolute RBF of the clamped kidneys after 48 h of reperfusion, as measured in the six rats subjected to a dose-response analysis, was 3.97 ± 0.43 mL/min. As in previous experiments in control rats (12), intrarenal serotonin elicited a biphasic response of RBF: a pronounced dose-dependent vasoconstriction was followed by a dose-independent vasodilation. The dose-response curve of RBF in ischemic kidneys was significantly different from the control curve at each dose of serotonin. Both the vasoconstrictive and vasodilating responses to serotonin were clearly enhanced after ischemia. As in control kidneys (12), ketanserin completely blocked the serotonin-induced vasoconstriction, but did not influence the vasodilatory component.

Table 2 represents the responses to serotonin at doses lowered to 80%, to compensate for the decrease of the basal RBF (4.92 ± 0.31 mL/min in control rats; see Reference 12). The serotonin-induced vascular responses to these lowered doses were still significantly enhanced in post-ischemic kidneys versus control kidneys.

The responses to intrarenally applied, augmenting doses of phenylephrine were not altered by ketanserin, demonstrating that the ketanserin treatment used is devoid of α-adrenolytic activity (Table 3).

Autoregulation Studies

Figure 1 represents the influence of ketanserin on the autoregulation curves in rats, 48 h after reperfusion. Before ischemia, normal autoregulation of RBF was observed in 12 rats. The linear regression line between 115 and 95 mm Hg in RPP is y = 0.005x + 4.32 (r² = 0.95). The difference in RBF at RPP of 115 mm Hg and 95 mm Hg, respectively, was only 2%, (4.86 ± 0.44 mL/min versus 4.77 ± 0.45 mL/min). A complete loss of autoregulation was observed in all animals 48 h after reperfusion. The slope of the regression line after 48 h between 115 and 75 mm Hg was significantly different from the slope of the control curve between 115 and 95 mm Hg. During ketanserin infusion, the RBF at 115 mm Hg had a tendency to increase (from 3.73 ± 0.75 mL/min before to 4.31 ± 0.80 mL/min during ketanserin infusion; not signifi-

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**TABLE 1.** Dose response to usual doses of intrarenal serotonin in control and post-ischemic kidneys, 48 h after reperfusiona

<table>
<thead>
<tr>
<th>Bolus Serotonin (μg)</th>
<th>0.3</th>
<th>0.5</th>
<th>0.75</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VC</td>
<td>VD</td>
<td>VC</td>
<td>VD</td>
</tr>
<tr>
<td>Control kidneys</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 ± 4</td>
<td>7 ± 2</td>
<td>21 ± 5</td>
<td>10 ± 3</td>
<td>33 ± 5</td>
</tr>
<tr>
<td>Post-Ischemic kidneys</td>
<td>23 ± 5</td>
<td>21 ± 4b</td>
<td>40 ± 6b</td>
<td>25 ± 2b</td>
</tr>
<tr>
<td>Post-ischemic kidneys (during ketanserin)</td>
<td>0b</td>
<td>19 ± 3b</td>
<td>0b</td>
<td>25 ± 5b</td>
</tr>
</tbody>
</table>

a The biphasic response is represented as a mean percentage of RBF decrease and a mean percentage of RBF increase versus the basal RBF (± SE). VC, vasoconstriction; VD, vasodilation.

b P < 0.05 versus control kidneys (N = 6).
Ketanserin and Repair of Autoregulation

TABLE 2. Dose response to usual doses of intrarenal serotonin in control and post-ischemic kidneys, 48 h after reperfusion

<table>
<thead>
<tr>
<th>Bolus Serotonin 20% Lowered (μg)</th>
<th>0.25</th>
<th>0.4</th>
<th>0.6</th>
<th>0.8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VC</td>
<td>VD</td>
<td>VC</td>
<td>VD</td>
</tr>
<tr>
<td>Control kidneys</td>
<td>5 ± 2</td>
<td>3 ± 1</td>
<td>17 ± 3</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Post-ischemic kidneys</td>
<td>17 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> The biphasic response is represented as a mean percentage of RBF decrease and a mean percentage of RBF increase versus the basal RBF (± SE). VC, vasoconstriction; VD, vasodilatation.

<sup>b</sup> P < 0.05 versus control kidneys (N = 6).

TABLE 3. Response of intrarenal bolus administration of phenylephrine on RBF (as a mean percentage of decrease of the basal RBF), before and during ketanserin administration in post-ischemic kidneys after 48 h and 7 days of reperfusion

<table>
<thead>
<tr>
<th>Bolus Phenylephrine (μg)</th>
<th>0.1</th>
<th>0.3</th>
<th>0.5</th>
<th>0.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 h reperfusion (N = 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before ketanserin</td>
<td>24.6 ± 5.5</td>
<td>50.7 ± 7.0</td>
<td>66.5 ± 7.8</td>
<td>76.7 ± 6.3</td>
</tr>
<tr>
<td>During ketanserin</td>
<td>21.2 ± 2.4</td>
<td>45.3 ± 4.3</td>
<td>70.9 ± 3.9</td>
<td>79.8 ± 4.7</td>
</tr>
<tr>
<td>7 days reperfusion (N = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before ketanserin</td>
<td>22.6 ± 2.8</td>
<td>43.3 ± 5.3</td>
<td>61.5 ± 6.3</td>
<td>69.4 ± 6.7</td>
</tr>
<tr>
<td>During ketanserin</td>
<td>24.9 ± 2.8</td>
<td>47.2 ± 4.7</td>
<td>58.7 ± 4.5</td>
<td>68.1 ± 5.0</td>
</tr>
</tbody>
</table>

Figure 1. □: autoregulation of RBF in pre-ischemic kidneys; △: RBF versus RPP in post-ischemic kidneys, 48 h of reperfusion; ○: reappearance of autoregulation of RBF in post-ischemic kidneys, 48 h of reperfusion, effect of ketanserin infusion (N = 15).

Figure 2 depicts the results after ketanserin addition, at 7 days of reperfusion. All six animals showed a complete loss of autoregulation. Infusion of ketanserin completely restored autoregulation of RBF in a similar way as in the experiments performed after 48 h of reperfusion. The regression line of RBF at RPP between 115 and 75 mm Hg after reperfusion was y = 0.022x + 1.505 (r² = 0.99). During ketanserin, the regression line between RPP 115 to 95 mm Hg was y = 0.009x + 3.068 (r² = 0.92). The differences in slopes between these regression lines were highly significant (P < 0.001).

Infusion of saline in these animals had no influence on the autoregulation capacity. The slope of the regression line during saline infusion is not different from the slope before infusion. The results obtained in cant (NSI). At a pressure of 95 mm Hg, RBF decreased only with 4.5% from the control value at 115 mm Hg. The slope of the regression line between 115 and 95 mm Hg during ketanserin (y = 0.010x + 3.176; r² = 0.981) is not significantly different from the slope of the pre-ischemic control regression line.

In another six animals, studied before ischemia and after 48 h of renal reperfusion, it is shown that the infusion of isotonic saline, instead of ketanserin, does not alter the linear regression line obtained before the saline infusion. The regression line is linear between 115 and 75 mm Hg; y = 0.028x + 0.502, (r² = 0.99). The slope of this regression line is significantly different from the slope before ischemia (P < 0.001; results not shown).
the four rats studied after 7 days of reflow were very similar to those studied after 48 h of reperfusion (results not shown).

Clearance Studies

At 48 h of reperfusion, 11 of the 15 post-ischemic kidneys were anuric. The GFR, diuresis, and FE\textsubscript{Na}, in the four non-anuric kidneys were 0.15 ± 0.04 mL/min, 1.52 ± 0.15 μL/min, and 0.14 ± 0.02% respectively. At 7 days of reperfusion, no post-ischemic kidneys were anuric. The ketanserin infusion lowered the MAP, but did not alter renal function significantly. The clearance results before and during ketanserin administration are summarized in Table 4.

Morphological Study

In lightmicroscopic sections, necrotic lesions are visible in the tunica media of arcuate and interlobular arteries of post-ischemic kidneys. These findings are confirmed electronmicroscopically: condensed nuclei are observed in small cortical arteries as an indication of myonecrosis (Figure 3).

DISCUSSION

The most interesting finding of this study is the beneficial effect of the S\textsubscript{2}-serotonin antagonist ketanserin on the autoregulation of RBF in the post-ischemic rat kidney: after ketanserin administration, this autoregulation reappeared almost perfectly at 48 h or at 7 days of renal reperfusion.

The normal renal autoregulatory response to a reduction in perfusion pressure is independent of renal innervation and accomplished by an intrinsic myogenic vasorelaxation (18) of predominantly the afferent arterioles, and, to a lesser extent, of the interlobular arterioles (19) and the arcuate arteries (20). Only in the later phase of autoregulation is tubuloglomerular feedback activated (21). The study presented here confirms previously published data that showed that the normal autoregulatory response of RBF to a reduction in RPP is lost after an unilateral renal ischemic insult (4–7,9,11). Several mechanisms can be offered to explain the loss of autoregulation of RBF. The most obvious explanation is a direct ischemic

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Ketanserin</th>
<th>During Ketanserin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>48 h of reperfusion (N = 15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFR (mL/min)</td>
<td>0.04 ± 0.02</td>
<td>1.61 ± 0.09</td>
</tr>
<tr>
<td>Diuresis (μL/min)</td>
<td>0.40 ± 0.18</td>
<td>7.09 ± 0.71</td>
</tr>
<tr>
<td>FE\textsubscript{Na} (%)</td>
<td>0.04 ± 0.01</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>113 ± 4</td>
<td></td>
</tr>
<tr>
<td>7 days of reperfusion (N = 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFR (mL/min)</td>
<td>0.27 ± 0.04</td>
<td>1.76 ± 0.16</td>
</tr>
<tr>
<td>Diuresis (μL/min)</td>
<td>2.01 ± 0.16</td>
<td>5.18 ± 0.48</td>
</tr>
<tr>
<td>FE\textsubscript{Na} (%)</td>
<td>2.9 ± 2.55 (N = 4)</td>
<td>0.04 ± 0.03 (N = 6)</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>108 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) \(P < 0.05\) before versus during ketanserin.
Ketanserin and Repair of Autoregulation

The majority of the clamped kidneys remained anuric, with the response in control kidneys. This increased during this acute administration of ketanserin. On the other hand, GFR, FENa and diuresis are not altered from the work of Endlich et al. (13). They visualized that ketanserin also has α-adrenergic blocking characteristics, besides its anti-serotonergic action. The dose applied in this study, however, is devoid of α-blocking actions: this dose does not reduce the RBF response to increasing doses of intrarenally administered phenylephrine, either in normal kidneys (12), or in post-ischemic kidneys (present study).

There is evidence that normal renal hemodynamics are influenced by serotonin. This is indicated by the fact that antagonism of serotonin S2-activity, by applying the same ketanserin-treatment as used in this study, increases RBF and GFR in rats. Ketanserin also maintains the autoregulation of RBF and GFR of the normal rat kidney in pressure ranges below the normal autoregulation area (12). The role of serotonin in normal renal hemodynamics can also be deduced from the work of Endlich et al. (13). They visualized serotonin effects on the preglomerular vessels of hydropnephrotic kidneys and showed that autoregulation of glomerular blood flow disappears after the addition of serotonin.

In the post-ischemic kidneys of this study, RBF shows a tendency to rise during the infusion with ketanserin, although not to a significant degree. On the other hand, GFR, FENa and diuresis are not altered during this acute administration of ketanserin. The majority of the clamped kidneys remained anuric, 48 h after reperfusion. GFR was restored to approximately 25 to 30% of normal at 1 wk of reperfusion, but renal function was not influenced by the acute administration of the S2 antagonist.

The fact that the lost renal autoregulation response in post-ischemic rats can be reversed by a 45 to 60-min infusion of an S2 antagonist might imply that the intrinsic myogenic response is not damaged, but only overwhelmed by a stronger vasoconstrictive influence. This contractile action might be the consequence of the enhanced serotonin S2 activity in the post-clamp kidneys. An argument in favor of this hypothesis is the increased vasoconstrictory response to serotonin in the post-ischemic kidneys, compared with the response in control kidneys. This increased vasoconstriction persisted even when serotonin doses were corrected for the RBF decrease after ischemia.

According to Endlich et al. (13), the constriction of the arcuate arteries in the presence of serotonin is the limiting factor for the adaptation of renal vascular resistance to a lowering of RPP in the non-ischemic kidney. The restrictive influence of serotonin on the autoregulation might thus be a result of its contractile effect on arcuate arteries. Yet, the fact that serotonin S2 antagonism prolongs the autoregulation reaction down to 70 to 75 mm Hg in a normal rat kidney (12) might be a result of the blockade of the predominant constriction of the arcuate arteries because of basal S2 receptor stimulation, inhibiting autoregulation at a RPP lower than the normal threshold of 90 to 95 mm Hg. The autoregulation is not prolonged down to 70 to 75 mm Hg by S2 antagonism in the post-ischemic kidneys, as observed before in control rat kidneys (12). It can be hypothesized that this is because of vascular smooth muscle cell damage. Damage was shown morphologically under the form of focal necrosis of the vascular smooth muscle cells in the arcuate and interlobular arteries.

It is interesting to note that besides the smooth muscle cell damage, endothelial injury can also play a role in the clamp model of renal ischemia (9,11). This could make the renal vessels more vulnerable to the contractile influence of serotonin. Almost all of the serotonin in the blood is contained in the platelets and is released when platelets aggregate (27). Vascular sensitivity to serotonin, which is released from aggregating platelet, is enhanced when the endothelium is damaged (28). Hypoxia-induced endothelial dysfunction is believed to augment the contractile effect of platelet products, further reducing the luminal area and increasing the obstruction to blood flow (24). Moreover, after ischemia (e.g., in patients with peripheral arterial obstructive diseases or after an acute myocardial infarction), a serotonin-induced platelet aggregation could be observed, suggesting a potential vicious circle of subsequent vasoconstrictions (29). However, in the kidney, the platelets might not be the only source of serotonin, as it is shown that non-blood-perfused kidneys (30) and proximal tubules (31) are able to synthetize this agent. From all of this, it is thus tempting to speculate on a serotonergic influence as a mechanism not only determining the pressure range of autoregulation of RBF in normal kidneys, but also contributing to loss of autoregulation in post-ischemic rat kidneys.

The serotonergic influence in post-ischemic kidneys could be more pronounced because of an upregulation of S2-receptor number and/or sensitivity, or to an increased concentration of serotonin in the post-ischemic kidney. Furthermore, it is known that threshold amounts of serotonin can enhance the vasoconstrictor response to other substances, e.g., norepinephrine, epinephrine, thromboxane A2, prostaglandin F2α, angiotensin II (32), and of endothelin (33), all agents that exert important effects on renal hemody-
In conclusion, the reappearance of the autoregulatory response in a post-ischemic kidney of a ketanserin-treated rat, together with an increase in serotonin-induced renal vascular resistance, suggests an enhanced serotonin $S_2$-receptor-mediated effect in intrarenal arteries after an ischemic insult. This suggestion implies that serotonin could play a key role in determining the effective renal autoregulatory range. In normal conditions, this might limit the expression of autoregulation at a RPP lower than 95 mm Hg; in the post-ischemic conditions of the present experiments, it could completely eliminate the autoregulation. The finding of a pivotal role played by serotonin in the expression of autoregulation in post-ischemic conditions could be of important clinical relevance. Serotonin $S_2$-antagonism might lead to an interruption of the chain of recurrent injuries because of the loss of autoregulation after an initial insult and/or to a quicker repair process.

ACKNOWLEDGMENTS

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