Dynamic characteristics and underlying mechanisms of renal blood flow autoregulation in the conscious dog

ARMIN JUST,1 HEIMO EHMKE,2 LIRA TOKTOMAMBETOVA,1 AND HARTMUT R. KIRCHHEIM1
1Institut für Physiologie und Pathophysiologie, Universität Heidelberg, D-69120 Heidelberg; and 2Institut für Physiologie, Universität Hamburg, D-20246 Hamburg, Germany

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Just, Armin, Heimo Ehmke, Lira Toktomambetova, and Hartmut R. Kirchheim. Dynamic characteristics and underlying mechanisms of renal blood flow autoregulation in the conscious dog. Am J Physiol Renal Physiol 280: F1062–F1071, 2001.—The time course of the autoregulatory response of renal blood flow (RBF) to a step increase in renal arterial pressure (RAP) was studied in conscious dogs. After RAP was reduced to 50 mmHg for 60 s, renal vascular resistance (RVR) decreased by 50%. When RAP was suddenly increased again, RVR returned to baseline with a characteristic time course (control; n = 15): within the first 10 s, it rose rapidly to 70% of baseline (response 1), thus already comprising 40% of the total RVR response. Thereafter, it increased at a much slower rate until it started to rise rapidly again at 20–30 s after the pressure step (response 2). After passing an overshoot of 117% at 43 s, RVR returned to baseline values. Similar responses were observed after RAP reduction for 5 min or after complete occlusions for 60 s. When tubuloglomerular feedback (TGF) was inhibited by furosemide (40 mg iv, n = 12), response 1 was enhanced, providing 60% of the total response, whereas response 2 was completely abolished. Instead, RVR slowly rose to reach the baseline at 60 s (response 3). The same pattern was observed when furosemide was given at a much higher dose (>600 mg iv; n = 6) or in combination with clamping of the plasma levels of nitric oxide (n = 6). In contrast to RVR, vascular resistance in the external iliac artery after a 60-s complete occlusion started to rise with a delay of 4 s and returned to baseline within 30 s. It is concluded that, in addition to the myogenic response and the TGF, a third regulatory mechanism significantly contributes to RBF autoregulation, independently of nitric oxide. The three mechanisms contribute about equally to resting RVR. The myogenic response is faster in the kidney than in the hindlimb.

renal hemodynamics; tubuloglomerular feedback; myogenic response

although many factors may modulate autoregulation of renal blood flow (RBF) (38), the basic function is presently believed to derive from only two underlying mechanisms: the myogenic response and tubuloglomerular feedback (TGF) (3, 23, 38, 48). However, there is no simple way of eliminating the myogenic response without affecting the TGF at the same time. Therefore, the contribution of the myogenic response has so far been deduced only by means of exclusion from the degree of autoregulation, which is left after blockade of the TGF by direct interruption (32, 44), clamping (42) of tubular flow, ureter occlusion (14, 41), or furosemide (2, 30, 37). Due to these experimental constraints, it cannot be excluded that the remnant autoregulation after blockade of the TGF comprises further regulatory mechanisms in addition to the myogenic response. Accordingly, it is not clear today whether mechanisms in addition to the myogenic response and the TGF contribute to the autoregulatory process.

The response times of the myogenic response and the TGF differ considerably from each other. Although in the kidney the myogenic response seems to reach its maximum effect within a few seconds after activation (10, 12, 21, 30, 53), TGF comprises a delay of 10–20 s (7, 13) and requires 1–2 min to achieve its full response (6, 7, 13). Therefore, it is possible to dissociate both mechanisms by their distinct temporal characteristics. This has been done successfully already by studying the transfer function (2, 12, 14, 21, 30) and by analyzing the autoregulatory response to a step increase in perfusion pressure (24, 40, 43, 45, 51, 53). Transfer function studies have shown that although active under physiological conditions (2, 12, 14, 21, 30), the TGF is not required to explain the limited autoregulatory strength, which is observed under these conditions (2, 14, 30). However, the transfer function does not allow for assessment of the actual contribution of the TGF under resting conditions. Although to some extent this would be possible by a step function analysis, the available studies have not directly made use of this feature and were limited to anesthetized animals (24, 43, 45, 49, 53) and the artificially perfused kidney (40, 51).

The response time of the myogenic response in renal vessels (3–10 s) is considerably faster than that in other vascular beds (30–120 s), such as those in skeletal muscle (16, 18, 25), brain (35, 39), and skin (15, 34). Although these temporal differences have been experimentally investigated by studying the renal and the mesenteric...
circulation (1) and discussed in a recent review (20), a direct comparison with other vascular beds to our knowledge has never been done, except for a cursory notion in the original work of Bayliss (5) and a step function analysis in artificially perfused organs (31).

Therefore, the purpose of the present study was threefold: 1) to investigate the step response of RBF autoregulation in the conscious dog to dissociate the myogenic response from the TGF and to test whether further mechanisms with still other response times may be involved; 2) to estimate the relative contribution of each mechanism to the autoregulatory adaptation of vascular tone under resting conditions; and 3) to compare the temporal pattern of the responses in the renal vessels to those in the hindlimb vasculature.

METHODS

In accordance with the national guidelines for the care and use of research animals (Reg.Präsr. Karlsruhe license 37-9185.81/156/96), 66 experiments were made in 18 conscious foxhounds (27–37 kg). The dogs were held under an artificial 12:12-h light-dark-cycle and were fed a standard dog diet (SSNIPP Spezialdiäten, Soest, Germany) with free access to tap water.

Chronic Instrumentation

The dogs were surgically prepared under sterile conditions. After premedication with atropine (0.5 mg sc; Braun, Melsungen, Germany) and propionylpromazine (Combelen, 0.5 mg/kg sc; Bayer, Leverkusen, Germany), anesthesia was induced by pentobarbital sodium (Nembutal, 20 mg/kg; Sanofi, Libourne Cedex, France) and maintained by halothane (Fluothane, 0.8–1.0%; Zeneca, Planckstadt, Germany) and N2O (0.5 l/min). The depth of anesthesia was checked by repeated testing of the eyelid reflex. At induction, masseter relaxation was also assessed. Via a left-flank incision, a polyurethane catheter was implanted into both the abdominal aorta and the left renal artery. An ultrasonic transit-time flow probe (type 6SS, Transonic, Ithaca, NY) was also mounted on the left renal artery. To allow a controlled reduction of renal arterial pressure, an inflatable vascular occluder was placed on the same artery distal to the flow probe. A Silastic catheter was implanted into the left renal vein for the infusion of drugs. Three dogs received no venous catheter. In these animals, the infusions were made through a cannula inserted into a limb vein at least 10 min before the experiment was started and removed afterward. To also assess the adaptive responses in the hindlimb circulation, in five dogs a flow probe (type 8RS, Transonic) and a vascular occluder were implanted on the left external iliac artery in a separate procedure through a transperitoneal approach after a midline abdominal incision under the same type of anesthesia. In three dogs, this was done after all experiments on the renal artery had been completed; the other two dogs were only subjected to the iliac implantation procedure without prior perirenal surgery. In these animals, the aortic catheter was implanted during the same iliac surgery. Catheters and cables were subcutaneously led to each animal’s neck, where they were exteriorized. At least 10 days were allowed for recovery before experiments were started. On the day of surgery and on days 3 and 6 thereafter, the dogs received a combination of benzylpenicillin and sulfatolamide (Tardomycel, 3 ml sc; Bayer). The catheter was flushed every second or third day and filled with a solution of heparin (1,700 IU/ml) in 0.9% saline. In some of the dogs this solution also contained cephtazidim (Fortum, 16 mg/ml; Glaxo, Bad Oldesloh, Germany) as described in more detail previously (4).

Measurements

All experiments were made between 7:30 and 11:30 AM while the dogs were resting on their right side as trained before. Arterial pressure in the aorta (AP) and in the renal artery (RAP) were measured from the two catheters by pressure transducers connected to amplifiers (Statham P23XL with Gould Pressure Processor, Gould, Valley View, OH). RBF was obtained by an external flowmeter (Transonic T106 or T108) connected to the implanted flow probe. The output of the flowmeter was low-pass filtered below 10 Hz by the built-in analog filter. In most of the experiments the air pressure used to control the vascular occluder was measured by a pressure transducer (BTE 4005G0, Sensor-Technics). All data were recorded continuously on a computer at 20 Hz (for more detail, see Refs. 28 and 30). This measurement equipment provided adequate dynamic characteristics for the purpose of the present study. The frequency response of the catheter-transducer system provides accurate transduction up to 1 Hz, and ~3-dB attenuation above 3 Hz (30), corresponding to a time constant of 53–159 ms. The frequency response of the RBF signal was limited only by the low-pass filter and the sampling frequency.

Protocols

Pressure reduction. To investigate the adaptive changes in renal vascular resistance (RVR) in response to a sudden increase in perfusion pressure, RAP was reduced to 50 mmHg for a duration of 60 s and was then suddenly restored to aortic AP. The reduction of RAP was achieved by inflation of the implanted occluder with room air controlled by hand with a 50-ml syringe while RAP was observed on a paper recorder (Gould 2600). This procedure is herein referred to as pressure reduction. Usually, RAP was restored almost immediately after reopening of the artery, at the latest with the next systole (see also Fig. 1A). If this was not the case, the response data were discarded. Under each experimental condition, at least three such pressure reductions were made, succeeding each other in intervals of 10 min, thus allowing intercalated recovery periods of 9 min. Periods of 4 min were also used in the case of high-dose furosemide to allow measurements at an early time point after the drug, and periods of 14 min were used after prolonged pressure reductions to allow longer recovery (see below). In eight dogs under control conditions, additional pressure reductions were conducted, during which the renal artery was completely occluded. In the case of technical insufficiencies of a pressure reduction or complete occlusion (e.g., failure to keep RAP constant, slow rise in RAP after reopening, movement or deep breath by the dog during the response), an additional reduction was made after the respective recovery time. At the end of each experiment, the renal artery was completely occluded for 30 s to obtain the zero-flow offset. In the case of iliac blood flow (IBF), the same protocol was followed, but because no catheter was available distal to the occluder, the artery was completely occluded for 60 s.

Experimental conditions. Each experiment, except for the complete occlusion and high-dose furosemide, was conducted on a separate day. The order of the experiments made in an individual dog was randomly assigned. After furosemide or Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME), 1 wk was allowed for recovery. Not all experiments were done in
Fig. 1. Original traces of renal arterial pressure (RAP), renal blood flow (RBF), and calculated renal vascular resistance (RVR) during a pressure reduction in the renal artery in a conscious dog. A: thick horizontal bars denoting the time periods over which data were averaged to derive baseline RVR (BASE), smallest RVR immediately after release of the pressure reduction (MIN), and RVR during the plateau phase (PLAT). B: RAP recorded at 20 Hz (solid lines) and smoothed by sliding average over 2 s (••••). C: RBF at 20 Hz (solid lines) and sliding average over 2 s (••••). D: RVR calculated from the smoothed data of RAP and RBF. RAP was artificially reduced to 50 mmHg between −60 and 0 s and was then suddenly released.

each individual, but a control experiment was done in every dog.

CONTROL. After a resting period of 10 min, three pressure reductions \( n = 15 \) were made in intervals of 10 min, i.e., 10, 20, and 30 min after the start of the recording (see also Fig. 2, Table 1).

Table 1. Experimental protocols

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Dog</th>
<th>( n )</th>
<th>( l )-NAME, min</th>
<th>SNAP, min</th>
<th>Furosemide, min</th>
<th>RAP Reductions, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1–15</td>
<td>15</td>
<td></td>
<td></td>
<td>10, 20, 30</td>
<td></td>
</tr>
<tr>
<td>Prolonged reduction</td>
<td>5, 6, 8, 9, 13–15</td>
<td>7</td>
<td></td>
<td></td>
<td>10, 25, 40</td>
<td></td>
</tr>
<tr>
<td>Complete occlusion</td>
<td>1–8</td>
<td>8</td>
<td></td>
<td></td>
<td>50, 65, 80</td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>1–3–8, 9, 11–14</td>
<td>12</td>
<td></td>
<td></td>
<td>25, 35, 45</td>
<td></td>
</tr>
<tr>
<td>Furosemide, high dose</td>
<td>9–14</td>
<td>6</td>
<td></td>
<td></td>
<td>50, 50, 55, 60, 65, 75, 85</td>
<td>115, 125, 135</td>
</tr>
</tbody>
</table>

Dog, identification nos. of individual dogs included in the respective experimental group; \( n \), number of dogs; \( l \)-NAME, \( N^{G} \)-nitro-\( l \)-arginine methyl ester; SNAP, \( S \)-nitroso-\( N \)-acetyl-\( l \)-penicillamine; RAP, renal arterial pressure; \( N \)-NO, nitric oxide. Furosemide refers to time points (in min) after the start of the recording, at which the respective drug was given or its infusion was started. RAP reductions refer to time points at which RAP reductions were applied.
occluded for 60 s (n = 5). This was repeated two to four times with recovery periods of 5–15 min. To test for possible metabolic effects, in two dogs additional occlusions were made in the same experiment, during which the artery was either completely occluded for 30 s or only partially occluded (IBF reduced but not stopped) for 25 s and then completely occluded for the final 5 s before being reopened. The order of the different types of pressure reduction was randomly assigned.

Data Analysis

The original 20-Hz data of RBF and RAP were smoothed by a sliding average over 40 values (2 s). RVR was calculated from the smoothed data of RBF and RAP by dividing (RAP – RAP0) through (RBF – RBF0), where RAP0 is the pressure at which RBF ceases, and RBF0 is the zero-flow offset. A value of 16 mmHg was assumed in all cases for RAP0, which is the average value found in the experiments in the present study with complete occlusion. In the first 27 experiments, in which the air pressure was not recorded, the exact time points of the beginning and end of the periods of reduced RAP were determined by visual inspection of the original 20-Hz RBF and RAP data. After an appropriate pressure transducer was made available in the following experiments, the 20-Hz signal of the air pressure in the occluder was used for automatic detection of the time points. On the basis of these time points, the time courses of the responses to all pressure reductions of an experiment were averaged. Trials with technical insufficiencies (see above) were discarded. For each experimental group a mean curve was calculated from the averaged time courses of all dogs. In addition, for each pressure reduction the following features of the time course of RVR were determined automatically (see Fig. 1A, thick horizontal bars) as follows: baseline (BASE in Fig. 1A) is the mean from the last 60 s before the pressure reduction; minimum resistance (MIN in Fig. 1A) is the mean from the initial 2.5 s after release; maximum of response 1 is the maximum found between 25 and 10 s after release after further smoothing by sliding average over 40 consecutive values; plateau (PLAT in Fig. 1A) is the mean from 10–25 s after release; and maximum of response 2 is the maximum found between 25 and 80 s after release after further smoothing by sliding average over 40 values. To estimate the relative contribution of the first and the second rise in RVR to the total autoregulatory response, the rise of RVR from MIN to PLAT was expressed as the percentage of the total response between MIN and BASE ($\text{PLAT}\% = \frac{\text{PLAT} - \text{MIN}}{\text{BASE} - \text{MIN}} \times 100$). The same normalization to the total response was made for all RVR values of the time courses of each individual pressure reduction (Fig. 3). Vascular resistance in the external iliac artery (IVR) was calculated from the smoothed data of aortic AP and IBF similar to the calculation of RVR, except that 0 mmHg was assumed for venous pressure [AP/(IBF – IBF0)]. Heart rate was determined off line, beat by beat from the 20-Hz AP signal.

The effects of the experimental interventions were determined by comparison with the respective values from the paired control experiments on the same individual. Statistical significance of these differences was tested by one-way ANOVA for repeated measures including all groups followed by a Student-Newman-Keuls test using SigmaStat 2.03 (SPSS, Chicago, IL). Normality was tested by a Kolmogorov-Smirnov test, equality of variances by a Levene median test. If one of the latter tests failed, Kruskal-Wallis one-way ANOVA on ranks was used followed by Dunn’s method of comparison. In all cases, a $P$ value of 0.05 was considered significant. All values are expressed as means ± SE.

RESULTS

The typical time course of RAP, RBF, and calculated RVR during one of the pressure reductions is depicted in Fig. 1. During the reduction in RAP (Fig. 1A), RBF (Fig. 1B) initially dropped considerably, but in the following time period it partially recovered. This autoregulatory behavior is reflected by an almost exponential decrease in RVR (Fig. 1C) during this period. With the subsequent step increase of RAP back to the systemic AP, RBF initially rose above the preocclusive level and then recovered from this reactive hyperemia in a characteristic manner.

Figure 2A shows the averaged time course of RVR of the control experiments from all 15 dogs. For the sake of simplicity, the data segment containing the RAP reduction has been eliminated in this and Figs. 3 and 4. Thus time 0 in these figures corresponds to the time of the release of the pressure reduction. Positive numbers on the time scale refer to the time after release, whereas negative numbers denote the time before the beginning of the pressure reduction. The relatively large variation in RVR among the dogs mainly derived from differences in the resting level of RBF (Table 2).
During the 60-s period of RAP reduction, RVR decreased from 0.33 to 0.17 mmHg·ml⁻¹·min⁻¹ (see Table 3). This corresponds to almost exactly one-half of the preocclusive value (51.0 ± 1.2%). From this level, RVR rose back to control in at least two successive steps: within the first 10 s it rapidly increased to 70.7 ± 1.3% of the preocclusive level (Fig. 2A). This is referred to in the following discussion as response 1. In some cases this response showed a small overshoot, but on average it reached only 98 ± 2% of the following plateau phase of RVR. During the subsequent 10–20 s, the rise continued at a much slower rate (Fig. 2A). This is referred to as the “plateau.” After a delay of 20–30 s after the pressure step (Fig. 2A), response 2 began. Because of the applied smoothing procedure, a more exact determination of the beginning of this response was not possible. Almost in every instance it displayed an overshoot, which, on average, reached 122 ± 4% of the preocclusive level and occurred at 45 ± 2 s after release of the occlusion.

To estimate a possible contribution of metabolic factors to this time course, additional experiments were conducted, during which the period of RAP reduction was either prolonged for 5 min or the renal artery was completely occluded for 60 s. After prolonged pressure reductions, the occurrence of the maximum of response 2 was significantly delayed by 20% (54 ± 4 vs. 45 ± 2 s, P < 0.01: Fig. 2B). After complete occlusion, the time course appeared similar (Fig. 2), although it took up to 5 min to reach the preocclusive level.

Fig. 4. Comparison of the responses of RVR to complete occlusion in the renal and hindlimb circulation (top traces). Averaged time courses of RVR in the external iliac artery (IVR) before and after complete occlusion for 60 s (thick line, n = 5) ± SE (dotted lines), after partial occlusion for 25 s followed by complete occlusion for 5 s (thin dotted lines, n = 2) are shown. Values are means ± SE. For comparison, the time course of RVR before and after complete occlusion for 60 s is shown (bottom traces). Thin solid line, mean (n = 5); dotted lines, SE. For the sake of comparability, RVR was investigated after inhibition of the tubuloglomerular feedback by furosemide (40 mg). All data are expressed as % respective baseline level.
were delayed, whereas the magnitude of its maximum (74 \(\pm\) 40–50 s after reopening; Fig. 2) as well as the maximum level (data not shown). In addition, both the onset (5 min for RVR to completely return to the baseline level (data not shown). In both experiments, the onset of RVR was delayed, whereas the magnitude of its maximum was slightly attenuated (88 \(\pm\) 2 vs. 119 \(\pm\) 3% of baseline RVR, \(P < 0.05\)).

Neither the smallest resistance at the end of the pressure reduction or complete occlusion (Table 3) nor the level of RVR during the plateau (Table 3) displayed any appreciable difference from the values found after 60 s of RAP reduction. These characteristics of the response, therefore, seem to be robust and largely independent of time and metabolic factors. Nevertheless, to keep confounding influences small, all other experiments were conducted with a pressure reduction for 60 s.

To test the possibility that capacitative effects due to pressure-induced changes in kidney volume might produce apparent changes in RVR, step changes in RAP were induced between 30 and 50 mmHg, i.e., below the pressure range of active autoregulation (data not shown). In this experiment, changes in RVR were seen after the pressure steps, compatible with capacitative effects. However, these were smaller than response 1 of RVR (~50%) and were completed after <20 s.

To investigate the contribution of the TGF to the observed response, experiments were conducted after administration of furosemide. Although mean RBF increased transiently after furosemide (data not shown), by the time of the pressure reductions (after 15 min) it had returned to its control level. Under these conditions, the minimum vascular resistance after the pressure reduction was neither in absolute values (Table 3) nor relative to the preocclusive level (56 ± 1 vs. 50 ± 1%), significantly different from the values obtained under control conditions. This indicates that the maximum capacity for pressure-dependent vasodilatation was significantly, but only slightly, attenuated. In contrast, the time course of RVR after release of the pressure reduction was significantly affected: although response 1 was enhanced so that RVR reached a higher level during the plateau (Table 3), response 2 was completely abolished (Fig. 3A).

To facilitate an estimation of the relative contribution of the different responses to the total regulatory change in RVR, it was normalized to the difference between the preocclusive baseline level and the minimum RVR after the pressure reduction (see also methods). This demonstrates that RVR reached 39 ± 2% of its total response during the plateau under control conditions, whereas it rose to 60 ± 3% after furosemide (Fig. 3A). This difference was highly significant (\(P < 0.001\)). Nevertheless, even after furosemide, RVR during the plateau was still clearly <100% of the total response (Fig. 3A), demonstrating that response 1 did not reach sufficient strength to elevate the plateau of RVR all the way back to the preocclusive level (Table 3, Fig. 3A). In contrast, after furosemide RVR further increased from the plateau in a slow rise to reach the baseline level after 68 ± 2 s, i.e., significantly later than under control conditions (45 ± 2 s, \(P < 0.001\)).

One simple explanation for these observations might be a dose of furosemide too small to completely eliminate the TGF. For this reason, experiments were made during which a much higher dose of furosemide was applied. Again, during the plateau, RVR did not reach the baseline level (Table 3) but only rose to 70 ± 5% of the total response compared with 46 ± 2% in the paired controls (\(P < 0.001\)). The same result was obtained when the pressure reductions were conducted at 5, 10, and 15 min after furosemide, when the plasma concentrations are expected to be highest (RVR during the plateau was 69 ± 6% of the total response). This was also similar to the plateau of RVR after the lower dose of furosemide in the same dogs (67 ± 4%).

These results indicated that not two but three regulatory mechanisms with different time courses contribute to RBF autoregulation. To investigate whether the slow rise in RVR after furosemide, i.e., the third mech-

Table 3. Renal vascular resistance before and during the response to the pressure reduction

<table>
<thead>
<tr>
<th>Experiment</th>
<th>(n)</th>
<th>BASE, mmHg·ml(^{-1})·min(^{-1})</th>
<th>MIN, mmHg·ml(^{-1})·min(^{-1})</th>
<th>PLAT, mmHg·ml(^{-1})·min(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>0.33 ± 0.03</td>
<td>0.17 ± 0.01</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>0.29 ± 0.02</td>
<td>0.15 ± 0.01</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>Prolonged reduction</td>
<td>7</td>
<td>0.30 ± 0.02</td>
<td>0.15 ± 0.01</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>0.36 ± 0.04</td>
<td>0.17 ± 0.01</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>Complete occlusion</td>
<td>8</td>
<td>0.36 ± 0.02</td>
<td>0.16 ± 0.01</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>0.34 ± 0.03</td>
<td>0.17 ± 0.02</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>Furosemide</td>
<td>12</td>
<td>0.36 ± 0.03</td>
<td>0.20 ± 0.02</td>
<td>0.29 ± 0.02</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0.27 ± 0.02</td>
<td>0.14 ± 0.01</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Furosemide high dose</td>
<td>6</td>
<td>0.28 ± 0.03</td>
<td>0.17 ± 0.01</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0.27 ± 0.02</td>
<td>0.14 ± 0.01</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Furosemide+NO clamp</td>
<td>6</td>
<td>0.34 ± 0.03</td>
<td>0.20 ± 0.02</td>
<td>0.28 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE. \(n\), No. of animals; BASE, mean renal vascular resistance (RVR) during 60 s before each pressure reduction; MIN, mean RVR during the initial 2.5 s after release of the reduction; PLAT, mean RVR between 10 and 25 s after release. No statistically significant differences among the experimental groups were detected by 1-way ANOVA (MIN) or Kruskal-Wallis 1-way ANOVA on ranks (BASE and PLAT).
anism, might derive from an adaptive release of endogenous NO, experiments were made during which plasma levels of NO were clamped. Under these conditions (Fig. 3C) there was a prominent augmentation of the overshoot of response 1 (143 ± 9 vs. 89 ± 3% of RVR at the plateau, *P* < 0.001). Nevertheless, RVR did not reach the baseline level during the plateau (Table 3, Fig. 3C) but rose to 61 ± 2% of the total response under furosemide compared with 67 ± 4% after furosemide alone and 46 ± 2% (*P* < 0.01) without administration of any drug, in the same group of dogs.

Additional experiments in three of the dogs showed that also after furosemide in combination with clamping of the plasma levels of ANG II, RVR during the plateau reached only 64 ± 6% of the total response, compared with 66 ± 7% after furosemide alone and 46 ± 4% without use of any drug in the same animals. Thus restoration of RVR during response 1 after the pressure step appears to be independent of changes in ANG II plasma levels.

The rapid time course of response 1 seemed surprising in view of the known response times in other vascular beds. Therefore, similar experiments were conducted on the external iliac artery in five dogs of the same strain. IVR was 0.58 ± 0.08 mmHg·ml⁻¹·min before the occlusion and fell during the occlusion to −30% of this baseline. From this level, it did not rise appreciably during the initial 4–6 s and then only slowly increased to reach the baseline level, but not before 20–30 s after release of the occlusion (Fig. 4, thick solid line). This time course was not substantially different if the occlusion lasted only 30 s (Fig. 4, thin solid line) and even if the artery was partially occluded for 25 s and then completely occluded for only 5 s (Fig. 4, thin dotted line). In contrast, in the response in the renal circulation after 60 s complete occlusion during furosemide, RVR fell to only 50% of the baseline value and then rapidly increased in response 1, already reaching its maximum 4–6 s after reopening of the artery.

**DISCUSSION**

The present study demonstrates the temporal characteristics of the myogenic response and TGF and their contribution to resting vascular tone in the kidney. In addition, the results strongly suggest the participation of a third, previously unrecognized mechanism in the autoregulation of RBF. Under resting conditions, −50% of the basal vascular tone is available for autoregulatory vasodilatation, to which all three mechanisms seem to contribute by approximately equal amounts. Finally, direct comparison with the autoregulatory response in the hindlimb circulation shows that the myogenic response in the kidney is substantially faster than that in skeletal muscle.

**Time Course**

The averaged time courses show that RVR adapts to a step increase in RAP in three steps with differing temporal patterns. At least the two faster responses are in perfect congruence with previous observations of the responses to a step rise in RAP (40, 43, 45, 51, 53), although in one of these studies (24) response 1 and thus the plateau were not overtly discernible.

Response 1, which reaches its maximum within the first 10 s after the pressure step, most probably comprises the myogenic response. Although no direct proof is possible, the most compelling evidence for this view is the pronounced similarity of the time course to that observed in rats after a rapid reduction of perirenal pressure (10). The vasoconstriction observed under the latter conditions can hardly be explained by mechanisms other than a myogenic response. The scarce occurrence of an overshoot of this response in our study is also in line with the findings by Clausen and co-workers (10). Because an overshoot can only be generated in a control system higher than first order, i.e., including a rate-sensitive component, the results suggest that the latter is not prominently active under these conditions. The same conclusion was drawn from the phase relationship of the transfer function between RAP and RBF (30) and is also in line with direct observations in isolated hamster cheek pouch arterioles (15). The overshoot was enhanced under furosemide, and even more so during additional clamping of the plasma levels of NO levels. Similar enhancements of the myogenic response and its dynamics have also been observed after furosemide (10) or NO synthase inhibition (26, 50).

Response 2, which started 20–30 s after the pressure step and almost always presented an overshoot at 40–50 s, not only largely resembles the temporal characteristics of the TGF observed in micropuncture experiments (6, 7, 13) but was also abolished by furosemide, which is known to block the TGF. The observed enhancement of this response after prolonged pressure reduction might be due to activation of the renin-angiotensin system with subsequent production of ANG II, which is known to augment the gain in TGF (38). The reduction of the maximum RVR of response 2 after complete occlusion might be explained by an additional depression of RVR by metabolic factors or by prolonged activation of a third response (response 3) induced by the complete occlusion. The slowing of this response might be caused by a more complete emptying of the tubuli than during the pressure reduction to 50 mmHg, leading to a further delay in the reinitiation of distal tubular flow.

After blocking of the TGF, response 3 becomes apparent, which comprises a slow rise in RVR, reaching the baseline level after 60 s. It is very unlikely that this represents a remnant function of the TGF due to an insufficient dosage of furosemide, because exactly the same changes in RVR were found after an extremely high dose of furosemide. Although the actual concentration of furosemide at the macula densa is not known, measurements of its concentration in the urine in similar experiments in a previous investigation from this laboratory (30) have suggested sufficient concentrations even after a dose of only 10 mg/kg. There was also no indication for any dose dependency with the
two doses of furosemide used in the present study. The observation that under control conditions RVR slowly increased during the plateau at an almost identical rate as the slow rise after furosemide suggests that the third mechanism is active also in the absence of furosemide, i.e., when the TGF is intact. Although it cannot be entirely excluded that part of response 3 may actually arise from capacitative effects due to slow changes in kidney volume, the observed changes in RVR after pressure steps between 30 and 50 mmHg do not seem large and slow enough to fully explain its time course. However, there is no simple way to assess capacitative effects in the normal pressure range. It seems noteworthy that a corresponding slow adaptation of RBF during the plateau phase under control conditions can also be detected in the data of previous publications (45, 53), although the authors did not explicitly mention this feature. Furthermore, in the transfer function between RAP and RBF, a small peak in the gain spectrum was observed, $\sim 0.016$ Hz (i.e., with a cycle length of 60 s) in the presence of furosemide (30), which may reflect the same phenomenon.

The nature of the mechanism of response 3 could not be clarified in the present work. A reasonable possibility would have been some action of endogenous NO, because a previous investigation had revealed an important modulatory influence of NO on humorally induced vasoconstrictor tone in the kidney (8), and other observations had suggested a role of phasic changes in this dilator substance in dynamic autoregulation of RBF (27). However, clamping of the plasma levels of NO did not induce any alteration of the level of RVR during the plateau compared with furosemide alone. In contrast, the time courses of RVR at least during the plateau phase were virtually superimposable after furosemide with and without the clamping of the plasma levels of NO. In addition, in a previous study (27) the clamping of plasma levels of NO by a very similar protocol indeed had an effect on the autoregulation of RBF. It should be noted that the present finding does not contradict a regulatory role for NO, operating in conjunction with the TGF (47) or mesangial chloride concentrations (46), because the present findings were made after blockade of the TGF by furosemide. In this respect, there is also no conflict with our previous finding of a phasic regulatory role of NO in RBF autoregulation (27). The present results rather suggest that the latter effect may be due to a TGF-associated mechanism.

Despite the small number of experiments and possibly confounding influences of kinins induced by the angiotensin-converting enzyme inhibitor, the experiments during clamping of ANG II plasma levels argue against ANG II as a mediator of the slow response of RVR. What the actual underlying causes are can only be speculated about. Possible factors include ATP, adenosine, or other purinergic derivatives (38), cytochrome P-450 metabolites (19), or intrarenally formed dopamine (33). Yet another possibility is that response 3 reflects a slow component of the myogenic response. Modifications in the kinetics of the myogenic response have been observed in the dependence of the rate of pressure change (15, 18) and after interference of the TGF (49).

Finally, the enhancement of response 1 after furosemide deserves some comment. It indicates an almost synchronous response of the whole preglomerular vasculature and also suggests that the dynamic properties of the myogenic response become altered in response to changes in the activity of the TGF. Changes in the kinetics of the myogenic response have also been reported from direct observations of afferent arterioles after elimination of the TGF by papillectomy (49). The fact that the augmentation of response 1 was even more pronounced after additional clamping of NO plasma levels is consistent with a role of shear stress-induced NO release for the reduction of resonance of the myogenic response (26).

**Quantitative Considerations**

During the period of pressure reduction, RVR fell by $\sim 50\%$ of the baseline level, independently of whether the pressure reduction was prolonged for 5 min or changed to a complete occlusion. This indicates that half of the resting vascular tone is due to autoregulation and is thus available for regulatory vasodilatation. This is in good agreement with the view that the glomeruli are situated midway between pre- and post-glomerular resistance and that the autoregulatory adaptation occurs predominantly in the preglomerular vessels (48), where it is brought about by all segments of this part of the renal vascular tree (9). It should be noted, however, that it cannot directly be inferred from the observation of the maximal vasodilatation in response to the pressure reduction well below the lower limit of autoregulation how efficiently this vasodilator capacity is employed for autoregulation within the autoregulatory pressure range. Therefore, the present results allow only conclusions regarding the total capacity of pressure-dependent vasodilatation from the basal vascular tone. In contrast to the autoregulatory efficiency (30), the vasodilator capacity was not significantly affected by furosemide in the present results. Because the regulatory mechanisms operate at different velocities, their relative contribution to the total amount of this autoregulation can be estimated, because of the following reasoning. Immediately after the pressure reduction, all three mechanisms contribute their maximum vasodilator signal. Subsequently, the fastest response adapts its influence to a vasoconstrictor influence according to the new pressure, while the other two continue to provide a vasodilator signal. Therefore, during the plateau phase, the relative contribution of the myogenic response can be estimated from the rise in RVR. This seems to amount to 40% of the total response. After blockade of the TGF, only the myogenic response and response 3 are operative. Therefore, the initial constrictor signal of the myogenic response is opposed only by the vasodilator signal of the more slowly responding response 3. Accordingly, the observations after furosemide suggest that, in the
absence of the TGF, the myogenic response contributes 74% to the total autoregulatory effect, whereas response 3 provides the remaining 26%. Under the assumption that only the myogenic response, but not the third mechanism, is enhanced after furosemide, it may be argued that the TGF contributes the remaining 34%. Although this estimation must remain open, the assumption receives some support from the observation that the slope of RVR during the plateau phase was almost identical to that with and without furosemide. Because the step responses were investigated between 50 mmHg and resting arterial pressure, it is possible that this mechanism is active only in this low-pressure range. In this respect, it might be important to an understanding of ischemic acute renal failure. Whether and to what extent this mechanism also contributes to autoregulation at physiological arterial pressure levels cannot be decided from the present data and have to await further study.

Comparison With Hindlimb Vasculature

The observed response time in the range of 30 s is very similar to those observed by other authors in visualized arterioles in the hindlimb muscle of cats (25) and dogs (16, 18) as well as in the cremaster muscle of rats (22, 36). The direct comparison in the present study of the vascular responses in the renal and the hindlimb circulation in animals of the same strain (in 3 of them, also in the same individuals) clearly shows that there is a distinct difference in the time course of the myogenic response in these two vascular beds. Teleologically, this could provide protection for the capillaries from rapid increases in pressure, which might be more important in the kidney than in skeletal muscle. The same consideration might apply to the mesenteric vasculature, where the myogenic response also seems to be faster than in muscular tissue (1). The fast response of the renal vessels does not seem to be an invariable feature, because other response times, more closely resembling the pattern in skeletal muscle arterioles, have also been observed in renal vessels (17, 52). In one of these cases, embryonic renal tissue had been transplanted to the hamster cheek pouch, the native vessels of which usually respond more slowly than original renal arterioles (17). It is therefore conceivable that the time course of the myogenic response may also be under the influence of tissue-specific paracrine signals.

Conclusions

Our data show that, in addition to the myogenic response and TGF, a third regulatory mechanism significantly contributes to the autoregulation of RBF, which is independent of NO and probably also of ANG II. Although our data do not provide direct evidence that this mechanism also contributes to autoregulation when the TGF is intact, the observation of the slow rise in RVR during the plateau under control conditions, which displayed almost the same slope as that after furosemide, supports this conclusion.

All three mechanisms seem to contribute equally to basal resting tone of the renal vasculature. This raises the question of whether the relative contribution might be altered, particularly under conditions known to be associated with a modulation of the gain in TGF, such as changes in blood volume or the activity of the renin-angiotensin system (38).

Furthermore, the comparison to the hindlimb circulation demonstrates that the myogenic response is substantially faster in the kidney than in the skeletal muscle. This difference may be relevant for the local regulation of blood flow and capillary pressure as well as for the variability of systemic arterial pressure.

Finally, the present results demonstrate a remarkable capacity for pressure-dependent adaptation of vascular tone in the vessels of the hindlimb, which seems to even exceed that in the kidney. Although this does not allow direct conclusions about the autoregulatory efficiency, the finding raises the suspicion that autoregulation in the skeletal muscle circulation might be more efficient than presently believed (11). The estimation of the autoregulatory strength in this vascular bed, however, will remain difficult, as noted by Jones and Berne (25) with respect to the ample distribution of vasodilator fibers in this region, which are in frequent use in the conscious animal (29).

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