The renin-angiotensin system and the third mechanism of renal blood flow autoregulation

Erdmann Seeliger,1 Thomas Wronski,1 Mechthild Ladwig,1 Leszek Dobrowolski,2 Torsten Vogel,1 Michael Godes,1 Pontus B. Persson,1 and Bert Flemming1

1Institut für Vegetative Physiologie, Charité-Universitätsmedizin Berlin, Campus Charité Mitte (CCM), Berlin, Germany; and 2Laboratory of Renal and Body Fluid Physiology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

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Seeliger E, Wronski T, Ladwig M, Dobrowolski L, Vogel T, Godes M, Persson PB, Flemming B. The renin-angiotensin system and the third mechanism of renal blood flow autoregulation. Am J Physiol Renal Physiol 296: F1334–F1345, 2009. First published April 1, 2009; doi:10.1152/ajprenal.90476.2008.—Autoregulation of renal blood flow comprises three mechanisms: the myogenic response (MR), the tubuloglomerular feedback (TGF), and a third mechanism (3M). The nature of 3M is unknown; it may be related to hypotensive resetting of autoregulation that probably relies on pressure-dependent stimulation of the renin-angiotensin system (RAS). Thus we used a normotensive angiotensin II clamp in anesthetized rats and studied autoregulation 1) by slow ramp-shaped reductions in renal perfusion pressure (RPP) followed by ramp-shaped RPP restorations and 2) by means of the step response technique: after 30 s of either total or partial supraparenal aortic occlusion, a step increase in RPP was made and the response of renal vascular conductance analyzed to assess the mechanisms’ strength and initial direction (vasodilation or constriction). The angiotensin clamp abolished the resetting of autoregulation during ramp-shaped RPP changes. Under control conditions, the initial TGF response was dilatory after total occlusions but constrictive after partial occlusions. The initial 3M response presented a mirror image to the TGF: it was constrictive after total but dilatory after partial occlusions. The angiotensin clamp suppressed the TGF and turned the initial 3M response following total occlusions into dilation. We conclude that 1) pressure-dependent RAS stimulation is a major cause behind hypotensive resetting of autoregulation, 2) TGF sensitivity strongly depends on pressure-dependent changes in RAS activity, 3) the 3M is modulated, but not mediated, by the RAS, and 4) the 3M acts as a counterbalance to the TGF and might possibly be related to the recently described connecting tubule glomerular feedback.

Changes in perfusion pressure alter local blood flow by a variety of mechanisms. On one hand, changes in pressure result in passive circular stretching or destretching of vessels and thus in parallel changes in blood flow. On the other hand, via various pathways, pressure changes evoke active responses of vascular smooth muscles, i.e., vasoconstriction or vasodilation. First, pressure-induced flow changes result in metabolic changes in the tissue that, in turn, impinge on vascular muscles, as exemplified by the phenomenon of reactive hyperemia (5, 6). Second, flow changes alter shear stress, which impacts on vascular muscles, e.g., via altered release of nitric oxide (8). Third, in many vascular beds the phenomenon of blood flow autoregulation is observed, i.e., the ability to dampen or even to abolish the effects that changes in perfusion pressure would otherwise inevitably have on flow (16, 35).

Compared with blood flow control of other vascular beds, control of renal blood flow (RBF) offers some striking differences. RBF appears less controlled by tissue metabolism; rather, renal metabolism is controlled by RBF (29, 39). Moreover, any change in renal perfusion pressure (RPP), via the kidney-specific mechanism of pressure-dependent renin release, inevitably changes the activity of the renin-angiotensin system (RAS) (52, 55), which via angiotensin II (ANG II) affects renal as well as nonrenal vessels. Another specific feature is the response to some vasoactive substances, e.g., adenosine is dilatory in most vascular beds but exerts a constrictive effect in the renal cortex (62). Finally, blood flow autoregulation in the kidney is intertwined with autoregulation of glomerular filtration rate (GFR). It has been known for a long time that two mechanisms contribute to RBF autoregulation: the myogenic response (MR) (1) and the tubuloglomerular feedback (TGF) (60). These mechanisms have distinct dynamic characteristics: the MR operates at a high frequency of 0.1–0.2 Hz and the TGF at a lower frequency of 0.02–0.04 Hz in rats (for review see Refs. 12, 22). In recent years, studies on dynamic properties of RBF control in dogs, rats, and mice provided evidence that there exists a third mechanism (3M) that operates at very low frequencies < 0.01 Hz (23–27, 65). These in vivo studies employed the step response technique, in which RPP is reduced for a short period of time and then rapidly restored according to a stepwise function and the time course of RBF restoration is analyzed.

The nature of the 3M is elusive. However, Just (22) and Cupples and Braam (12), considering its very low operating frequency, recently hypothesized that the 3M may be related to the “slow component of RBF autoregulation” that Cupples had described in 1993 (10). Because the 1993 experiments were done at hypotensive levels of RPP, it was concluded that the “slow component” represents the same phenomenon as the hypotensive resetting of autoregulation (10, 20, 56). This resetting is known from studies that employed slow staircase-wise or slow rampwise reductions in RPP followed by slow RPP restorations to obtain “classical” autoregulation curves and found a marked RBF hysteresis: at the same RPP, lower RBF values are seen on the upward limb than on the downward limb of the curve (10, 14, 56). The degree of this hysteresis depends on the rate of RPP change and, thus, on the duration of the hypotensive period (14). Therefore, elevated ANG II level resulting from pressure-driven increase in renin release is
the prime candidate responsible for the hysteresis. In accordance with this, angiotensin-converting enzyme inhibition (ACE-I) (10, 20) or a normotensive ANG II clamp (56) abolished the hypertensive resetting, and ACE-I abolished the “slow component” (10).

Here we tested the hypothesis that the 3M of RBF autoregulation is equivalent to Cupples’ “slow component,” and thus relies on pressure-dependent changes in RAS activity. We utilized a normotensive ANG II clamp to prevent endogenous RAS changes from exerting effects via altered ANG II levels. First, we studied the role of endogenous RAS changes in the hysteresis in overall autoregulation. Second, using a previously developed mathematical analysis of the step response of RBF that allows us to determine the strength of the 3M as well as those of the MR and the TGF in vivo (65), we determined the role of the RAS for the three mechanisms. We also gained insight into RBF control by comparing it with a nonrenal vascular bed mainly perfusing skeletal muscles, the hindquarter (HQ) circulation.

METHODS

The experiments were performed on male 3- to 4-mo-old Wistar rats of 250- to 300-g body mass (BfR, Berlin, Germany). The rats received a standard diet; 12 h before the experiment, they were deprived of food but allowed free access to tap water. The investigations were approved by the Berlin government in accordance with the German Animal Protection Law.

Surgical procedures, measurements, and interventions. The rats were anesthetized by intraperitoneal injection of urethane solution (20% in water, 6 ml/kg body mass; Sigma-Aldrich, Steinheim, Germany). Rats were positioned on a heated table (39°C). A tracheal cannula was inserted for spontaneous breathing. A catheter was advanced into a jugular vein. The abdominal cavity was opened by a midventral incision. An inflatable cuff was placed around the suprarenal aorta just below the junction of the superior mesenteric artery. A catheter was inserted into the femoral artery with its tip toward the aorta and then connected to a pressure transducer and an amplifier (Gould, Valley View, OH). Two ultrasound transit time flow probes (1RB, Transonic Systems, Ithaca, NY) were positioned by use of micromanipulators, one around the left renal artery and the other around the abdominal aorta below the origin of the renal arteries. Arterial blood pressure (aortic pressure below the aortic cuff), RBF (left kidney), and HQ blood flow (HQF) were recorded continuously. After analog-to-digital conversion, the data were stored online with a low-pass element of the second order (PT2) (13, 34, 41). Compared with the theoretical background with equations and examples in the supplementary material for the present article, the time course of blood flow restoration is also influenced by the actual time course of pressure restoration, which in vivo may deviate from the ideal rectangular step increase. Thus we analyzed the step response of the aortic cuff release of the aortic cuff to bring PP back to baseline values measured immediately before the start of the respective intervention.

Analysis of step response. The rapid release of the aortic cuff results in an immediate stepwise increase in PP. In turn, RBF and HQF are restored from their values achieved during the occlusion to stable levels close to their preocclusion values. The time course of the RBF and HQF transients, i.e., the step response, depends on the dynamic properties of the respective vasculature.

The mathematical analysis of the step response has been described in our previous paper (65). Readers who are not familiar with technical control theory can find an extended description of the theoretical background with equations and examples in the supplementary material for the present article. The time course of blood flow is approximated by a function according to

$$u_{out} = u_{in} \cdot k \cdot [1 + A e^{-\omega t} \cos(\omega t + \phi)]$$

in which $u_{out}$ denotes the output signal (RBF or HQF), $u_{in}$ the input signal (PP), $k$ the proportional control factor, $A$ the amplitude, $\omega$ the angular frequency, $\delta$ the dampening, and $\phi$ the phase angle.

In engineering, this function is used to describe the step response of a low-pass element of the second order (PT2) (13, 34, 41). Compared with mathematical models of the first order that have been used to analyze blood flow step responses (22, 63), this model has the advantage of accurately describing both oscillations and aperiodic time courses according to the actual value of $\delta$. Equation 1 describes the superposition of a time-independent proportional term $u_{in}k$ and a time-dependent term $u_{in}kAe^{-\omega t} \cos(\omega t + \phi)$ that tapers off with time.

The time course of blood flow restoration is also influenced by the actual time course of pressure restoration, which in vivo may deviate from the ideal rectangular step increase. Thus we analyzed the step response of conductances instead of that of the flows to eliminate this potential source of error (51). The time course of conductances ($C$) is approximated by

$$C = \frac{u_{out}}{u_{in}} = k \cdot [1 + A e^{-\omega t} \cos(\omega t + \phi)]$$

The proportional control factor $k$ is calculated by division of the last values $u_{out}/u_{in}$ after flow restoration, i.e., after achieving a new stable level (34). The other parameters of Eq. 2 are calculated by an iterative

Subsequently, an intravenous bolus of 1 ml/kg body mass of isotonic saline containing 0.33 mmol/l captopril (Sigma-Aldrich) was given. After recordings of blood pressure, RBF, and HQF, a normotensive ANG II clamp was made as described by Leong et al. (31). Isotonic saline containing 0.4 $\mu$mol/l ANG II (Sigma-Aldrich) was mixed 1:1 with the above captopril solution. The mixed solution was continuously infused intravenously at individual rates that were adjusted to the actual blood pressure in order to restore it to the individual rat’s pressure value immediately before the pressure decline induced by the captopril bolus. Average infusion rates of the captopril-ANG II solution were 4 ml/h. Fifteen minutes after a steady state was achieved, the interventions to determine the characteristics of renal and HQ vasculature were repeated.

Data analysis. The original data were averaged by sliding average with a window size of 1 s. To distinguish changes in flow brought about by changes of pressure via passive vessel distension from those brought about by vasomotor actions, conductances (the inverse of resistance) were calculated by dividing flow data by the present PP (i.e., arterial pressure; venous pressure was not taken into account); renal vascular conductance (RC) was calculated by dividing RBF by present PP and conductance of the HQ vascular bed (HQC) by dividing HQF by present PP. Relative values of PP, RBF, HQF, RC, and HQC were obtained by relating the absolute data to the absolute baseline values measured immediately before the start of the respective intervention.
least-squares fitting procedure described in detail in Ref. 65. The residuum of the first fitting process provides the starting point for the second fitting, and so on. This procedure reaches coefficients of determination \( r^2 \) of \(-0.98\) after three iterations (65). As a result of the three subsequent fitting procedures, up to three separate eigenoscillations are detected, each of which is characterized by certain values of angular frequency, amplitude, dampening, and phase angle. By the parameter angular frequency (or oscillation period), the observed oscillation can be ascribed to certain regulatory mechanisms (for details see below). The oscillation’s amplitude characterizes the strength of the respective mechanism (65).

Compared with our previous article (65), the further analysis was modified as follows. To allow direct comparison between the amplitudes following total occlusion and those following partial occlusion, i.e., to account for the different height of the actuating pressure step, the amplitudes were normalized by division with the individual step’s height (relative to baseline PP). The amplitudes were also normalized with regard to the time of the pressure step. Comparison of phase angles as obtained by every fitting procedure revealed that the maximum deflection occurred at different times. Therefore, the deflection at the moment of the step was calculated by multiplying the amplitude with \( \cos \varphi \).

While we had presented data as the square of the amplitudes (a measure of energy content) (65), we now present the unsquared amplitudes. The advantage is that, in addition to the amplitude’s absolute value that indicates the strength of a given mechanism, the algebraic sign of the amplitude of the oscillation’s first half-wave is denoted, which allows interpretation in terms of the initial direction of a given mechanism’s effect, i.e., initial vasoconstriction or vasodilation.

By use of the parameter oscillation period the oscillations in RC are ascribed to one of the three renal autoregulatory mechanisms (65). Because our mathematical analysis has not hitherto been applied to the HQ circulation, we first set out to determine whether the HQ step response contains oscillations and, if so, to distinguish classes of oscillation periods to ascribe them to known mechanisms of pressure-dependent control of this circulation. An extra set of experiments was run with a total of 40 rats (including the rats of the present protocol) that were prepared as described above, and the renal and HQ step responses following 30 s of total and partial occlusions were obtained. Figure 1 depicts the obtained oscillation amplitudes as histograms, i.e., as a function of the oscillations’ period duration, whereby the individual rats’ amplitudes for a given period duration were added up. In both vascular beds, oscillations with a period duration of less than 3 s were excluded, because they reflect passive distension of vessels due to the rapid increase in PP (10, 24).

In the HQ circulation, high-frequency oscillations were detected ranging from 3- to 27-s period duration after total occlusion (Fig. 1B) and from 3 to 18 s after partial occlusion (Fig. 1D). In the intermediate range (27- to 45-s period duration), no oscillations were detected. Very low-frequency oscillations ranging from 78- to 200-s period duration were observed following partial occlusion.
duration after total occlusion (Fig. 1B) and from 45 to 170 s after partial occlusion (Fig. 1D) were also detected. Thus for the further analysis we presumed that two mechanisms contribute to pressure-dependent response of the HQ circulation, and set the ranges of period durations to 3–30 s and 30–200 s, respectively. On the basis of the frequency of the MR in the HQ reported by Just and Arendshorst (24) and the negative amplitudes that indicate initial vasoconstriction, we assume that the high-frequency oscillations represent the MR. The initial vasodilatory effect (positive amplitudes) and the very low frequency of the other oscillations lead us to assume that these oscillations represent the metabolic component of pressure-dependent HQF control that is related to the phenomenon of reactive hyperemia (5, 6, 28).

The results for the renal circulation corroborate our previous results after total occlusion (65) (Fig. 1A). There are three ranges of period durations, which have been assigned to the renal mechanisms by means of comparison with known operating frequencies of the renal MR and TGF as well as by pharmacological interventions (65). The high-frequency oscillations (3- to 20-s period duration) represent the MR, the low-frequency oscillations (20- to 60-s period duration) the TGF, and the very low-frequency oscillations (60- to 200-s period duration) the 3M. After partial occlusion (Fig. 1C), oscillations of the same ranges of period durations can be distinguished, whereby the amplitudes of the TGF and 3M changed their respective algebraic sign.

We had previously studied the step response following total occlusions only (65), and the ascription of low-frequency oscillations (20- to 60-s period duration) to the TGF was supported by the result that these oscillations were suppressed by furosemide. Because the ranges of period durations of the individual mechanisms could possibly vary when the preceding occlusion is partial instead of total, we ran an extra set of experiments. Fourteen rats were prepared as described above, furosemide (Lasix, Hoechst, Germany; iv bolus of 20 mg in 2 ml) was administered, and the renal step response following partial as well as total occlusions was analyzed. As shown by Fig. 2, furosemide almost completely abolished the amplitudes in the TGF-related range from 20- to 60-s period duration following both partial and total occlusions. Furosemide did not reduce the amounts of amplitudes with period duration longer than 60 s (3M-related oscillations) and shorter than 20 s (MR-related oscillations). The distribution of amplitudes within the 3M range and within the MR range was comparable to those under control conditions. Intriguingly, the direction of the 3M-related amplitudes following total occlusions was changed by furosemide (see below). Taken together, the oscillations of 20- to 60-s period duration can be ascribed to the TGF following both total and partial occlusions, and the ranges of period duration do not vary when the preceding occlusion is partial instead of total.

Statistical analysis. Comparisons were made by the Friedman test for paired data and the Kruskall-Wallis test for unpaired data, with $P < 0.05$ to indicate significance. Data are given as means ± SE.

RESULTS

Absolute values of mean arterial blood pressure, RBF, HQF, RC, and HQC under control conditions, after bolus injection of captopril, and after initiation of the normotensive ANG II clamp are shown in Table 1. Captopril decreased blood pressure, and the ANG II clamp restored it to control values. Captopril left RBF and HQF unchanged; thus both RC and HQC increased. The ANG II clamp restored both RC and HQC.

The effects of the normotensive ANG II clamp on the relationship between PP and RC as well as HQC during ramp-shaped reductions in PP followed by ramp-shaped PP

![Fig. 2](http://ajprenal.physiology.org/)

Fig. 2. Histograms of amplitudes as a function of the oscillations’ period duration (from 0 to 200 s with a class width of 3 s) obtained by mathematical analysis of the step response of renal conductance following partial (left) or total (right) occlusion during control conditions (top, $n = 13$) and after administration of furosemide (bottom, $n = 14$). The individual rats’ amplitudes for a given period duration are added up. Because the analysis is based on relative conductance changes, the sum of amplitudes is without dimension.
restorations are depicted in Fig. 3. Under control conditions, relative RC increased, i.e., vessels dilated with decreasing PP during the decremental ramp, reaching peak values of −1.1 at PP slightly below 60 mmHg (Fig. 3A). With further PP reduction, RC declined. During the incremental ramp, RC changes showed roughly a reversed image. However, RC values at corresponding PP values were lower during the upward than the downward leg. Thus the incremental-to-decremental conductance ratio (hysteresis) was below unity throughout the PP range of 60–90 mmHg.

Under control conditions, HQ vessels dilated with decreasing PP during the decremental ramp, reaching peak values of −1.2 at PP of −40 mmHg (Fig. 3C). During the incremental ramp, HQC changes showed roughly a reversed image. However, HQC at corresponding PP values was higher during the upward than the downward leg; thus the ratio was above unity in the PP range of 50–90 mmHg (Fig. 3D). The normotensive ANG II clamp tended to strengthen HQ vasodilation during both the decremental and the incremental ramp (Fig. 3C), and the ratio was unchanged compared with control conditions (Fig. 3D).

The time courses of RC and HQC following step increases in PP are depicted in Fig. 4. Conductance values dramatically increased within the first second after release of the cuff, obviously because of passive distension of the vessels. Within <3 s, distensions were uniformly counteracted by vasoconstriction. The degree of this counteraction, and thus the magnitude and duration of the initial overshoot, varied with the different conditions and vascular beds. The same applied to the later conductance changes that ultimately restored preocclusion conductance levels again. Remarkably, however, oscillations are easily visible in the time courses following both total and partial occlusions. This is in accord with many previous step response experiments (2, 24, 26, 37, 48, 51, 65).

![Fig. 3. Relative changes of renal conductance (A) and hindquarter conductance (C) during ramp-shaped reductions in perfusion pressure (decremental ramp, DR) followed by ramp-shaped pressure restorations (incremental ramp, IR) with intact renin-angiotensin system (RAS) (control) and during a normotensive angiotensin II (ANG II) clamp. Note that the duration of the period with lowered perfusion pressure (beginning at control pressure, ending with complete restoration of control pressure) was ~270 s. Relative conductance values greater than unity indicate active vasodilation in the face of lowered pressure. Hysteresis, i.e., different relative conductance at corresponding pressure during IR compared with DR, is depicted by the conductance ratio IR/DR (B and D). Values are means ± SE, n = 13 rats. *P < 0.05, control vs. ANG II clamp; **P < 0.05, control vs. 1.0 (hysteresis); ***P < 0.05, ANG II clamp vs. 1.0 (hysteresis).](image-url)
Under control conditions, the renal step response following total occlusions differed markedly from that following partial occlusions (Fig. 4A). The passive distension was pronounced after partial occlusion, whereas the initial vasoconstriction was pronounced after total occlusion. Remarkably, the subsequent vasomotor actions following partial occlusion were an almost perfect mirror image of those following total occlusion. The normotensive ANG II clamp altered the RC time courses (Fig. 4C). The passive distension following total occlusions became markedly greater than under control conditions. Also, the differences of the subsequent vasomotor actions between total and partial occlusions became smaller.

Comparison with the time course of HQC restoration (Fig. 4B) reveals striking differences. After total occlusions, the passive distension was much larger in the HQ, and the initial vasoconstriction was followed by a massive long-lasting dilation. In contrast to the renal vessels, the passive distension was markedly smaller after partial than total occlusions. The initial HQ constriction following partial occlusions was followed by a slow dilation that eventually restored preocclusion HQC. The changes induced by the ANG II clamp appeared small (Fig. 4D): after partial occlusions, passive distension was enlarged and the dilatory response restored preocclusion HQC faster than under control conditions.

Our mathematical analysis reveals that the time courses of RC and HQC restoration reflect the lumped responses of up to three mechanisms that display dampened oscillations superimposed on exponential time courses. The individual mechanisms’ dampened oscillations must not be confused with visible oscillations in the time courses of RC and HQC (Fig. 4). If oscillations of different period durations, amplitudes, dampings, and phase angles are superimposed, their wave interferences must generate a lumped time course that makes it unfeasible to unequivocally distinguish the individual mechanisms’ action at one point of time by visual inspection. By means of our mathematical analysis, these oscillations are detected and their individual characteristics quantified. The effects of the normotensive ANG II clamp on the amplitudes and period durations of the respective mechanisms of pressure-dependent blood flow control are depicted in Figs. 5 and 6. It should be noted that the amplitudes signify the strength and direction of the initial vasomotor action exerted by a given mechanism. In addition, the amplitudes are standardized with regard to the individual height of the actuating step in PP; thus in Fig. 5 the relative strength of a given mechanism is depicted to allow comparison between total and partial occlusions. For example, the standardized amplitudes of renal MR (Fig. 5A) do not differ between total and partial occlusions, although the absolute degree of the initial vasoconstriction is larger after total occlusion (Fig. 4A).

Under control conditions, the standardized amplitudes of high-frequency oscillations that represent the renal MR did not differ between total and partial occlusions. This mechanism’s initial effect was uniformly vasoconstrictive (Fig. 5A).
in striking contrast to the amplitudes of low-frequency oscillations representing the TGF: total occlusions resulted in a dilatory initial effect of TGF, whereas partial occlusions resulted in a constrictive initial effect. Intriguingly, the very low-frequency oscillations representing 3M presented a mirror image of the TGF: whereas total occlusions resulted in a constrictive initial effect, partial occlusions resulted in a dilatory initial effect. These opposing effects of total versus partial occlusion also become obvious from the histograms (comparison between Fig. 1, A and C).

The normotensive ANG II clamp tended to increase renal MR amplitudes after total occlusions; thus they were larger than after partial occlusions (Fig. 5C). Remarkably, the TGF amplitudes were almost completely abolished after both total and partial occlusions. Furthermore, the initial effect of 3M that was constrictive after total occlusions under control conditions became dilatory; this dilatory response even tended to be stronger than that after partial occlusions. There were no significant changes in the average period duration within one of the ranges of renal oscillation periods that would indicate altered time constants of a given mechanism, neither regarding the preceding PP reductions nor because of the ANG II clamp (Fig. 6, A and C).

Intriguingly, the results obtained during the ANG II clamp are comparable with those obtained with furosemide (Supplemental Fig. S2). Furosemide almost completely suppressed the TGF amplitudes after both partial and total occlusions. In addition, furosemide also turned the initial 3M response following total occlusions into constriction.

Under control conditions, the amplitudes of high-frequency oscillations representing the MR of the HQ were similar after total and partial occlusions. This mechanism’s initial effect was uniformly constrictive (Fig. 5B). The amplitudes of very low-frequency oscillations probably representing the metabolic component were smaller after partial than total occlusions. Its initial effect was uniformly dilatory.

In contrast to the renal vasculature, the average period duration of the HQ MR was lower after partial than total occlusion. Also, the average period duration of the metabolic component’s oscillations was strikingly lower after partial than total occlusion. These differential effects of total versus partial occlusion also become obvious from the histograms (comparison between Fig. 1, B and D). Compared with control conditions, the only significant change induced by the normotensive ANG II clamp in the HQ was that the MR became significantly stronger after partial than total occlusions.

**DISCUSSION**

The present study confirms that pressure-dependent RAS stimulation is the major cause behind hypotensive resetting of RBF autoregulation. Moreover, the study yielded four salient results. First, whether the initial responses of both the TGF and the 3M to a step increase in pressure are constrictive or dilatory
depends on the preceding hypotension, i.e., partial occlusions elicit responses reciprocal to those of total occlusions. Second, the sensitivity of the TGF response does not depend just on the presence of physiological ANG II levels but on pressure-dependent changes in RAS activity. Third, although the 3M is not mediated by the RAS, it is modulated by it. Fourth, the initial vasomotor actions elicited by the TGF and the 3M are opposing, indicating a concerted action of the autoregulatory mechanisms.

The pathways by which ANG II impacts on RBF are multiple. ANG II directly constricts preglomerular and postglomerular vessels (30, 66), thereby potentially changing filtered NaCl load, which in turn will change distal load including macula densa load, i.e., the tubular TGF signal (49). Moreover, ANG II appears to be involved in the paracrine signaling of TGF as a cofactor to adenosine and ATP (21, 50). ANG II also increases NaCl resorption; thus distal NaCl load is altered even at constant filtered load. This action increases metabolism and decreases tissue oxygen tension (40). Both TGF and 3M have been hypothesized to serve the purpose of balancing metabolic and oxygen demands with delivery and, thus, RBF (7, 39, 42, 65). These various actions of ANG II on RBF control are further confounded by hypertensive or hypotensive side effects in experiments with either ANG II infusion or ACE-I alone. To exclude this influence, and, in particular, to prevent endogenous RAS changes from exerting effects by altered ANG II levels, we used the normotensive ANG II clamp. As shown by Table 1, the clamp not only restored pressure to the normotensive level before ACE-I, but also restored renal and HQ conductances.

Perfect autoregulation, i.e., unchanged flow in the face of pressure changes, is limited to a certain pressure range. In many studies, a pressure value that signifies a “lower limit of autoregulation” is determined (for critical discussion of the methods see Refs. 12, 14, 61). Unfortunately, the term implies that autoregulatory activity is nonexisting below the limit. This is not the case: here, the flow response to pressure changes is a mixture of passive diameter changes and counteracting yet not counterbalancing vasomotor activity (3, 9, 14). The presence of the active component, i.e., autoregulatory activity, is unequivocal when conductance increases in the face of decreasing pressure. In accord with previous results (14), we find relative RC greater than unity at pressures far below the “lower limit” during rampwise RPP reduction (Fig. 3). For these reasons, we regard autoregulation as all vasomotor activity that counteracts passive stretching or destretching of vessels.

Hypotensive resetting and hysteresis in RBF and HQF control. Under control conditions, i.e., when pressure-dependent changes in RAS activity can exert their effects, RC at corresponding RPP was lower during the incremental than the decremental ramp. Thus a hysteresis-like resetting is observed, with a weakened dilatory response on the upward limb (Fig. 3). The normotensive ANG II clamp markedly enhanced the dilatory response during the upward limb such that the dilation became even stronger than on the downward limb. This corroborates the results of an elaborate study by Sorensen et al.
(56) in which a similar ANG II clamp not only abolished the resetting induced by 10–15 min of RPP reduction to 65 mmHg, but improved RBF autoregulation. As shown by these findings, pressure-dependent RAS stimulation is the major cause behind hypotensive resetting of RBF autoregulation.

It may appear surprising that overall autoregulation is not compromised by the ANG II clamp, even though the intervention almost abolished one of the autoregulatory mechanisms, the TGF (Fig. 5). Genetic ablation of adenosine type 1 receptors and furosemide administration that also result in TGF inhibition have been shown to diminish autoregulatory effectiveness (18, 24, 25). However, our result is in line with previous reports: ACE-I, ANG II infusion, and ANG II receptor antagonists, all of which affect the responsiveness of TGF, do not compromise steady-state autoregulation (for review see Ref. 22).

In line with previous results (16, 35), the HQ circulation displays autoregulation (Fig. 3), despite the fact that in the present setting it is also exposed to the effects of renal pressure-dependent RAS stimulation. In contrast to the kidney, dilation was stronger during the upward than the downward limb. Because both vasoconstrictive ANG II and local dilatory metabolites will increase with time, the positive hysteresis indicates the stronger effect of the latter, resulting in reactive hyperemia (5, 6, 28). The renal circulation revealed a positive hysteresis only during the ANG II clamp. This indicates that renal dilatory mechanisms are masked by stronger effects of pressure-dependent RAS stimulation. It should be noted that ACE-I also inhibits the breakdown of kinins, and bradykinin may cause efferent arteriolar dilatation; however, the quantitative contribution of this effect appears to be small (58).

Studies in hydropnephrotic rat kidneys found the MR to pressure oscillations of 1–6 Hz uniformly vasoconstrictive and the magnitude of the response dependent on peak pressure (33). Our aortic cuff reduces not only mean pressure but also pulse pressure amplitude; thus the dilatory responses to RPP reductions may be slightly overestimated.

Myogenic response in RBF and HQF control. The MR of renal vessels was uniform with regard to its vasoconstrictive initial action and its average period duration (Figs. 5 and 6). Also, the relative strength of the MR, i.e., normalized to the pressure steps’ height (Fig. 5), was similar after partial and total occlusions. With the ANG II clamp, the enlarged distension following total occlusions (Fig. 4) was counteracted by a larger absolute MR constriction; even the relative strength of the initial response was greater (Fig. 5). Comparison with the HQ circulation reveals a remarkable difference: the average period duration of the HQ MR was longer after total compared with partial occlusions (Fig. 6). This is in line with studies that found the speed of the MR in nonrenal vascular beds including the HQ lower than that of renal MR when large pressure steps were studied (1, 26), but similar at smaller pressure steps around normal pressures (24). One reason behind this difference could be involvement of vessels of different diameters (22). Another explanation is that different mechanisms may be involved in the MR of these vascular beds. The present finding that the strength of HQ MR was greater after partial than total occlusions during the ANG II clamp, while renal MR showed an opposite effect (Fig. 5), and the earlier finding that renal but not HQ MR is susceptible to nitric oxide synthase inhibition (24) support this concept.

Tubuloglomerular feedback in RBF control. It is well known that the TGF displays spontaneous oscillations that are also present in RBF (4, 22, 32, 44). Analysis of RBF oscillations, mostly done in the frequency domain by transfer function analysis between RBF and RPP [with naturally occurring or forced oscillations of RPP as actuating variable (19, 64)], has enabled insight into dynamic autoregulation and the contributing mechanisms (12, 22). Our approach to analyze, in the time domain, oscillations that are actuated by stepwise RPP changes yielded interesting new data on the TGF.

With intact RAS, the initial TGF response to step increases in RPP was dilatory after total occlusions but constrictive after partial occlusions (Figs. 1 and 5). The cause behind the opposing TGF responses is unclear. Considering that the input signal for TGF is tubular NaCl concentration at the macula densa (50), and that glomerular filtration ceases at RPP <40 mmHg (53), we speculate that the following different chains of events result in these TGF responses. During total occlusion (RPP nearly zero) filtration, and thus tubular flow, ceases, whereas NaCl resorption continues. This should lower NaCl concentration in premacula tubular fluid. When RPP is restored, tubular fluid with lowered NaCl concentration reaches the macula densa and promptly results in a dilatory initial TGF response. The present partial occlusions, on the other hand, reduced RPP to 60 mmHg, i.e., higher than the minimum pressure necessary to drive filtration. During this occlusion, tubular flow is probably slow but continuous and NaCl resorption continues, resulting in lower NaCl concentration. This fluid with lowered NaCl concentration reaches the macula densa already during the occlusion (as opposed to the conditions during total occlusions) and causes a dilatory TGF response. When RPP is restored, tubular flow is normalized; thus tubular fluid with normal NaCl concentration reaches the macula densa again, which promptly results in a constrictive initial TGF response.

It should be noted in this context that RBF autoregulation and GFR autoregulation share several characteristics and mechanisms, but are not identical. In particular, the TGF response does not rely solely on the vasomotor response of afferent arterioles but can involve efferent arterioles (46, 59), and efferent arteriole vasomotor action has opposing effects on RBF and GFR. Thus the opposing initial TGF responses of RBF following total versus partial occlusions do not necessarily apply to the TGF responses of GFR.

The normotensive ANG II clamp almost abolished the TGF response (Fig. 5). As recently reviewed (21, 50), the TGF (assessed by micropuncture techniques) is abolished in mice lacking either adenosine type 1 receptors or P2X1 purinergic receptors, pointing at adenosine and ATP as required constituents of the paracrine signaling pathway. Remarkably, the TGF response was also found largely absent in mice with deletions of ANG II type 1 receptors or ACE (50). This is in line with previous studies in rats in which actions of the RAS were abrogated by ACE-I or ANG II antagonists (for references see Ref. 22). Conversely, increasing ANG II to hypertensive levels markedly augments the TGF response (27, 36, 49). ANG II-adenosine interactions at the level of vascular smooth muscles are not fully understood (30). However, there appears to be consensus that ANG II is a mandatory cofactor of the action of adenosine and ATP in TGF (50). Our finding that the normotensive ANG II clamp almost abolished the TGF indicates that...
the role of the RAS in TGF exceeds that of ANG II as a permissive factor: the presence of ANG II at normotensive levels did not suffice to elicit a normal TGF response. Rather, pressure-dependent changes in RAS activity, in the present setting, increased ANG II levels induced by pressure-driven renin stimulation, appear to be mandatory for the TGF response. Whether this relies on direct ANG II effects on preglomerular vessels is unclear; it is also conceivable that effects on postglomerular vessels or indirect effects via tubular resorption play a role.

From a recent study with differential genetic ablation of ACE in mice (17), it was concluded that local ANG II generation by renal tissue ACE is required for TGF. The massive TGF augmentation by pressure-dependent RAS changes observed in our experiments (Fig. 5) most probably relies on locally generated ANG II. Peti-Peterdi and coworkers (43) demonstrated that renin release from afferent arterioles as stimulated by reduction in perfusion pressure or β-adrenergic agents takes <1 s. Not only the release but also the enzymatic activity of the released renin, i.e., generation of ANG I, was visualized in real time. Intriguingly, a significant portion of renin is released into the interstitial side of the juxtaglomerular apparatus. Furthermore, these authors found that a dynamic fluid flow exists in the juxtaglomerular interstitium that originates from ultrafiltration of blood plasma at the fenestrated portions of afferent arterioles. This fluid flow takes <5 s to reach the macula densa cells (47). Thus, in our experiments with 30 s of reductions in perfusion pressure, there was ample time for intrarenally generated ANG II to induce the TGF augmentation. A contribution of systemically generated ANG II to this effect during partial occlusions is theoretically possible. However, within the time frame of 30 s, the contribution of systemically generated ANG II is most probably much smaller than that of locally generated ANG II. Because ACE-I may not abolish intrarenal ANG II generation completely (38), the impact of pressure-dependent changes in RAS activity is possibly even underestimated in our results.

Third mechanism in RBF control and metabolic component of HQF control. The hypothesis that the 3M may rely on ANG II was deduced by combining two concepts, i.e., that hypotensive resetting of RBF autoregulation relies on pressure-dependent RAS stimulation and that the 3M may represent the same phenomenon as the hypotensive resetting (12, 22). The present results clearly indicate that the first concept is correct (Fig. 3), but the second is not; pressure-dependent RAS changes do not mediate the 3M (Fig. 5). The 3M was discovered by time-domain analyses of RBF restoration following pressure steps to or from hypotensive RPP levels (23–27, 65). Accordingly, the hypothesis was formed that the 3M may rely on ANG II. However, the 3M has recently also been detected at normal pressures by means of a high-resolution spectral analysis in the frequency domain (54).

With uncompromised RAS, the direction of the initial 3M response to step increases in RPP clearly depended on the preceding hypotension (Figs. 1 and 5): it was constrictive after total but dilatory after partial occlusion. The 3M response was thus a mirror image of the TGF response. One may therefore speculate that the signal eliciting the 3M response may also be of tubular origin. The very recent finding that, besides the classical macula densa-mediated TGF, there exists another tubuloglomerular signaling (45) may support this idea. Because the connecting tubule (CNT) in the outer renal cortex returns to the glomerulus and contacts the afferent arteriole, Ren et al. (45) perfused afferent arterioles and adherent CNT simultaneously and found that increasing the NaCl concentration perfusing the CNT diluted preconstricted afferent arterioles. This is the exact opposite of the effect that increasing the NaCl concentration at the macula densa exerts on afferent arterioles. The authors called this signaling “connecting tubule glomerular feedback” (CTGF). Since the average passage time of tubular fluid from the glomerulus to the CNT (57) is comparable to one-half of the period duration of the 3M oscillations, and because the initial vasomotor actions of the 3M were reciprocal to those of the classical TGF, the 3M may be the same as the CTGF. If this were true, the reason for the opposing initial 3M responses following total versus partial occlusions can be explained by different tubular NaCl load at the CNT, as above elaborated for the opposing TGF responses.

An alternative concept of 3M is based on the hypothesis that mechanisms of RBF autoregulation serve to balance metabolic and oxygen demands with oxygen delivery (7, 39, 42, 65). This was one of the reasons we used both total and partial occlusions, and why we compared the renal responses with those of the HQ, a vascular bed known to elicit metabolite-driven reactive hyperemia (28). The initial response of the very low-frequency mechanism in the HQ was uniformly dilatory (Fig. 5). The dilatory initial response of the renal 3M following partial occlusions (Fig. 5), in conjunction with its very low frequency that corresponds with that of the metabolic component of the HQ, suggests that the 3M may also rely on accumulation of dilatory metabolites. The fact that the 3M’s initial response was constrictive after total occlusions but dilatory after partial occlusions, does not, prima facie, speak in favor of a metabolic nature of 3M, because one would expect the accumulation of dilatory metabolites to be greater during total occlusions. However, any effect of metabolite accumulation may have been overridden by the massive pressure-dependent renin stimulation, and thus ANG II accumulation, that is inevitably induced by total occlusions. In line with this notion, the 3M became dilatory after total occlusions during the ANG II clamp. In addition, adenosine could play a role in the reciprocal initial responses following partial versus total occlusions. In nonrenal vascular beds, adenosine is one of the dilatory metabolites that mediate reactive hyperemia. In the renal cortex, adenosine has constrictive effects, and its accumulation is one of the causes behind the short-lasting renal vasoconstriction (‘reactive hypoxemia’) following total occlusions (62). It is conceivable that after partial occlusions far less adenosine is accumulated; thus yet-unidentified metabolites with renal dilatory effects may have caused the initial dilatory response of 3M. It must be pointed out that, should the accumulation of metabolites result in preglomerular dilation, not just RBF would increase, but also GFR and, in turn, the tubular workload. In case of an ongoing metabolite-driven dilation and a lack of counteraction by other mechanisms, e.g., by the TGF, this would result in a deleterious positive feedback. However, the observed effect on RBF could also rely on efferent dilation, which would decrease GFR and the tubular workload, thus having a “beneficial” effect.

Together, the present results clearly indicate that 3M is not mediated by ANG II, but ANG II modulates the response.
Whether the renal 3M relies on metabolic signals, tubular signals, or both remains to be clarified.

**Myogenic response, tubuloglomerular feedback, and third mechanism: interplay and concerted action.** The mechanisms of RBF autoregulation share a common effector, the vascular smooth muscle cells, and interactions among the mechanisms have been detected by studies in both the time and frequency domains (for review see Refs. 11, 12). These studies focused on interactions between the MR and the TGF. A recent spectral analysis in the frequency domain (54) also described phenomena that point toward an interaction of the 3M with the MR and the TGF. From our analysis of pressure step-induced oscillations in the time domain, an interplay of the three mechanisms becomes apparent, which may even indicate a concerted action.

An eye-catching phenomenon in the amplitudes of renal mechanisms (Fig. 5A) is that the direction of the initial 3M response is opposite to the direction of the initial TGF response: after total occlusions the initial TGF response is dilatory and that of the 3M constricive; after partial occlusions the TGF is constricive and the 3M dilatory. This suggests that the 3M exerts some kind of “corrective” action to that of the TGF. Taking into consideration that the MR’s initial constricive response was stronger after total than after partial occlusion (note that Fig. 5 depicts the relative strength normalized to the pressure step’s height), it appears that the TGF, on its part, takes some “corrective” action on that of the MR. Thus, after total occlusion, the massive constricive MR is to some degree offset by the dilatory initial TGF response, and the constricive initial response of the 3M provides final adjustment. Accordingly, the weaker constricive MR following partial occlusion is supported by the constricive TGF, and the dilatory response of the 3M results in amediantory fine tuning. The results during the ANG II clamp (Fig. 5C) as well as those with furosemide (Supplemental Fig. S2) support this notion: with the abolishment of the TGF, the amplitudes of the uniformly dilatory 3M were an almost perfect mirror image of that of the MR. This suggests that in the face of the lack of correction by the TGF, the 3M alone takes on the burden of amediantory action.

**Conclusions.** Redundant mechanisms control RBF in order to maintain the balance between filtration and resorption. Moreover, specific metabolic demands of specific kidney regions must be satisfied. In consequence, at least three mechanisms participate in the fine-tuning of RBF. The recently discovered 3M can act as a buffer for the TGF and, in case the TGF is not operative, even for the MR. This study also adds to our understanding of the RAS with regard to TGF mediation: clamping the RAS suppresses the TGF response, indicating that the RAS is more than a mere permissive factor.

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**REFERENCES**


