Acute Vasodepressor Effect in Normotensive Rats Following Extracorporal Perfusion of the Declipped Kidney of Two-Kidney, One Clip Hypertensive Rats

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SUMMARY Experiments were performed to determine the extent to which the rapid normalization of arterial pressure after renal declipping in chronic two-kidney, one clip renal hypertensive rats (RHR) is caused by the release of depressor agents from the kidney. Such a phenomenon would then represent a "hypotensive disturbance" of a structurally already adapted hypertensive cardiovascular system, rather than a true normalization of cardiovascular homeostasis. Consequently a marked pressure fall was expected to occur also in a normotensive rat when exposed to the venous effluent from an extracorporally perfused, acutely declipped kidney of a chronically hypertensive RHR.

In 10 experiments, isolated clipped RHR kidneys were perfused via an extracorporeal pump perfusion circuit from conscious normotensive rats. Mean arterial pressure (MAP), heart rate (HR), and cardiac output (CO) were followed in these normotensive rats, and the values remained largely unchanged as long as the renal artery clip was left in place. Upon sudden removal of the renal clip, with the renal perfusion pressure being maintained at the hypertensive level, the MAP of the conscious, normotensive rats fell from 127 ± 2 to 85 ± 7 mm Hg (p < 0.001) within 20 minutes, and decreased to 69 ± 7 mm Hg in 1 hour. However, the HR remained almost unchanged, despite the marked pressure fall due to a reduction of CO from 29.1 ± 2.5 to 14.7 ± 2.5 ml/min/100 g body weight (p < 0.001). Similar extracorporal perfusions of isolated normotensive kidneys did not cause any significant changes in either MAP, HR, or CO. These findings suggest the release of powerful depressor agents from an acutely declipped kidney. The depressor agents may, at least in part, elicit their hypotensive effects by an action on the central nervous system, since the drop in pressure was not accompanied by any reflex tachycardia, which occurs when similar pressure reductions are induced by blood loss or vasodilator substances. (Hypertension 4 (suppl II): II-101-II-105, 1982)

KEY WORDS • renal antihypertensive substances • kidney cross-perfusion • reversal of renal hypertension

In rats with chronic two-kidney, one clip renal hypertension (RHR), mean arterial pressure (MAP) usually declines toward normotensive levels a few hours after declipping of the renal artery. This rapid MAP normalization has often been assumed to show how an elimination of the provoked renal "pressor influence" permits a prompt return to normal cardiovascular homeostasis. However, in this situation RHR still exhibits considerable structural adaptation of the high-pressure cardio-vascular compartments, including the precapillary resistance vessels. These vessels are altered in such a way that a higher flow resistance than normal is maintained for a given smooth muscle activity, termed "structural auto-regulation." Therefore, the prompt MAP normalization in declipped RHR would suggest an acute induction of a subnormal vascular tone and/or a reduced cardiac output. In other words, the situation may rather represent a relative hypotension in a still principally hypertensive cardiovascular system, a situation similar to that which occurs when depressor influences in normotensive rats reduce MAP well below the ordinary 100-120 mm Hg.

It should be emphasized that the mere withdrawal of a prolonged vasoconstrictor influence, to which vascular smooth muscle function has become adapted, might per se lead to a considerable reduction of
smooth muscle tone, the reverse of the well-known supersensitivity displayed by cells after prolonged deprivation of their ordinary stimuli. Additionally, it is of great interest in this context that Muirhead and coworkers have observed a release of depressor substances from the medullar interstitial cells in their renal declipping experiments. In general agreement with this latter finding is the recent observation of Bing et al. that RHR with chemically "medullec-tomized" kidneys showed only modest MAP reductions upon renal artery declipping.

We considered it of interest to explore to what extent normotensive rats, lacking all the secondary changes of functional and structural nature accompanying chronic hypertension, exhibit hypotension and altered cardiovascular homeostasis when extracorporally perfusing chronically clipped RHR kidneys and these kidneys are acutely declipped.

Methods

Rat Preparation

Renal hypertension was induced in male Wistar rats, weighing 180 g, by placing a silver clip around the left renal artery, leaving the right kidney intact. The left kidneys from 10 such renal hypertensive rats (RHR, duration at least 3 weeks; MAP > 150 mm Hg) with clips on their left renal arteries were subsequently used for acute extracorporal perfusions by conscious normotensive rats. The left kidney from six male normotensive Wistar rats, 3–4 months of age, served as controls to evaluate the effect of extracorporal perfusion of an isolated, otherwise normal kidney. Ten male adult Wistar rats were used for these extracorporal perfusions, and recordings were taken of their mean arterial pressure (MAP), heart rate (HR), and cardiac output (CO).

On the morning of the experimental day the normotensive Wistar rats, used for the extracorporal perfusions of the isolated kidneys, were catheterized (PE-50) in the tail and left carotid arteries and in the right jugular vein during methohexital anesthesia (Brietal, 75 mg/kg i.p.). The carotid artery and jugular vein catheters were exteriorized at the back of the neck, allowing the rat to move freely in its small box (220 × 160 mm) where it was placed for recovery and for all remaining experimental procedures. The tail artery catheter was connected via a Statham P23 DC pressure transducer to a Grass polygraph (model 7B) for continuous recording of MAP and HR (fig. 1).

Measurement of Cardiac Output

The CO was measured using a dye dilution technique in microscale. Cardiogreen solution (0.04 ml) was injected from a step-dispenser syringe via a T-cannula into the jugular vein. Arterial blood was drawn at a constant speed (0.9 ml/min) from the carotid artery catheter through a low volume (0.05 ml) densitometer, from which the dye dilution curve could be recorded on a Servogor recorder. The drawn blood was then reinjected to the rat. The CO could be calculated from the recorded dye dilution curves. To perform the CO measurements, the renal perfusion had to be turned off for about 30 seconds.

Kidney Preparation

During anesthesia with sodium pentobarbital (Mebumal, 50 mg/kg i.p.) the left kidney of either the RHR, or of the normotensive rat (i.e., the control kidney), was gently isolated and cannulated (PE-90) via the distal aorta in a retrograde direction, though with the normal renal blood perfusion still going. The arterial clip was left in place for the RHR kidney. After isolation, the left renal vein was cannulated (PE-90), and with no time for circulatory arrest the kidney perfusion was changed from the native one to that of the extracorporal perfusion circuit, already connected to the awake normotensive rat. Immediately after this, the aorta was ligated also proximally to the left renal artery, leaving the by now perfused kidney in situ. Then the RHR or the normotensive rat, from which the kidney was taken, was killed by heart extirpation.

Extracorporal Circulation

This circuit was first primed with about 10 ml of blood from other Wistar rats, after which the carotid artery catheter and jugular vein catheter of the rat to be studied was connected to the circuit. The arterial pressure in this extracorporal circuit was, however, set by a small pump and recorded via a T-cannula just prior to the isolated perfused kidney. The flow rate was estimated by a drop-recorder unit. In this way the perfusion pressure to the isolated, clipped kidney could be maintained by pump adjustments at the hypertensive level originally present in the RHR. The blood passed through a small heating chamber prior to the perfused kidney, and the temperature of the isolated kidney was kept at 38°C. The kidney was placed slightly above the perfusing rat to keep renal venous outflow pressure low, and the venous effluent reached the rat via the jugular vein catheter. This catheter was wide enough to keep venous pressure in the perfused kidney very low.

Experimental Protocol

The conscious normotensive rat, habituated to its cage, appeared entirely undisturbed also when connected to the extracorporal perfusion circuit. When this was connected, control measurements of MAP, HR, and CO were performed. Changes of pressure and flow by way of pump adjustments in the extracorporal circuit, over the range used when a kidney also was incorporated, did not significantly affect the measured hemodynamic parameters in the rat.

In six of the 10 experiments, an isolated control kidney, taken from normotensive rats, was first connected to the extracorporal circuit and perfused for at least 60 minutes at a pressure around 110 mm Hg.
Thereafter this kidney was exchanged for the isolated but still clipped RHR kidney, which up to that moment had been perfused by the RHR. Now the circuit perfusion pressure was reset by the pump to about 150 mm Hg to mimic the in vivo MAP situation in RHR. First, after a control period of 30 minutes of extracorporeal perfusion, the renal artery clip was removed by careful dissection during continuous renal perfusion, with the pump adjusted to maintain the perfusion pressure for the acutely declipped kidney at the previous hypertensive level. The hemodynamics of the conscious normotensive rat were then followed for more than 1 hour after removal of the renal artery clip. Thus, in four of the 10 experiments, there was no initial period with perfusion of a normotensive control kidney. In this way it could be clarified whether a reduction of the total time of extracorporeal circulation, preceding the declipping, affected the results.

**Statistical Evaluations**

Values are presented as means ± se. Student's group comparison $t$ test was used to evaluate the significance of the difference between groups. A pairing design $t$ test was used to evaluate changes within each group. A $p$ value of less than 0.05 was considered significant.

**Results**

Extracorporeal perfusion of control kidneys from normotensive Wistar rats ($n = 6$) did not significantly change the hemodynamics of the conscious normotensive rats, compared with the situation when only the artificial circuit was perfused. The MAP, initially averaging 114 ± 2 mm Hg, increased slightly in these rats, to 118 ± 4 mm Hg after 20 minutes and to 128 ± 6 mm Hg after 60 minutes of perfusion of the normotensive control kidney (fig. 2). The HR, initially 384 ± 20 beats/min, remained essentially unchanged after 60 minutes of perfusion, 398 ± 24 beats/min.

After change to the clipped RHR kidney, the MAP started at 125 ± 4 mm Hg and remained largely unchanged, being 127 ± 2 mm Hg at the moment when the renal artery clip was removed. However, within a few minutes after the clip was removed, the MAP of the conscious rats started to fall to 102 ± 10 mm Hg after 10 minutes ($p < 0.01$) and to 85 ± 7 mm Hg ($p < 0.001$) after 20 minutes. During this MAP decline HR did not increase, being 399 ± 12 beats/min initially and 399 ± 20 beats/min after 20 minutes of declipped perfusion. However, the CO, which was 29.1 ± 2.5 ml/min/100 g b.w. just prior to declipping, fell to 14.7 ± 2.5 ml/min/100 g b.w. 20 minutes after removal of the clip.
Discussion

The results show that the MAP decreases markedly in normotensive rats during extracorporeal perfusion of acutely declipped kidneys from chronic, two-kidney one clip renal hypertensive rats (RHR). This MAP reduction cannot be explained by the circulation procedure per se, since no MAP reduction was seen when the rats perfused only the extracorporeal circuit, even beyond the flow rates seen after declipping, or when a normal kidney was included in this circuit. The results confirm earlier findings by Muirhead and co-workers** that depressor substances are released in hemodynamically significant amounts from acutely declipped kidneys upon reversal of renal hypertension.

Hallbäck-Nordlander et al.* noted that the rapid MAP reduction following declipping in RHR, with normalization of MAP within 24 hours, was not followed by any signs of reflex tachycardia. Such was also the case in the present study when awake normotensive rats perfused declipped RHR kidneys. This may indicate that the released renal substances exert their depressor action, at least in part, via the central nervous system. MAP reductions of a similar magnitude (by blood loss, for example) in the awake donor rats regularly produced considerable tachycardia. Furthermore, it was noted that the rats appeared to be "sedated" after perfusing the declipped kidneys for some time, judging from their behavior, but hardly so after perfusing normal kidneys. Such behavioral changes call for more precise measurements, however.

It is therefore possible that central sympathetic control is also interfered with by the substances released from the declipped kidney, which may importantly reduce venous return, for example. At least at one stage of the hypotensive response (20 minutes after declipping) CO was so markedly reduced that peripheral resistance was then actually increased by 25% to 30%. Therefore, it may be that the released depressor substances also exert negative inotropic effects on the myocardium.

Although CO fell in the normotensive rats used in the present study, Hallbäck-Nordlander and co-workers* found that the CO remained largely unchanged in the intact declipped RHR, where total peripheral resistance decreased markedly, instead. Apart from the different time relationships, arrangements, and protocols in the two studies, which might considerably influence the hemodynamic events, the divergent response patterns may to some extent be due also to the hypertrophied heart in RHR. The marked afterload reduction may here particularly tend to increase stroke volume, which may be more or less offset if concomitant venous return and hence preload also falls, so that the CO is better maintained in RHR. Such complex interactions, which certainly call for additional experimental analyses, may partly explain the divergent changes in CO and stroke volume in normotensive and renal hypertensive rats.

The present results clearly indicate that the rapid MAP normalization in declipped RHR is at least ini-

During more prolonged perfusion of the declipped kidney, MAP showed a further slow reduction, being 69 ± 7 mm Hg after 60 minutes of perfusion, while HR remained unchanged at 382 ± 12 beats/min. If, after a period of perfusion, the declipped perfused kidney was disconnected from the rat, pressure was regained quite slowly, and even more slowly so after about an hour of perfusion, suggesting a long-lasting action and/or an accumulation of depressor substances from the perfused, declipped RHR kidney in the rat. The depressor response pattern, occurring in the four conscious rats that were directly coupled to an acutely declipped RHR kidney, was the same as in the six rats that for a 60-minute period first perfused a normotensive kidney before being connected to the RHR kidney.
tially a matter of a functional hypotension in a principally hypertensive cardiovascular system. It is thus clear from earlier studies\(^1\)\(^2\) that both heart and systemic resistance vessels in chronic RHR hypertension have become structurally redesigned in response to the raised MAP equilibrium, and remain so for at least the first few days after declipping. For example, the resistance vessels in chronic RHR hypertension have narrowed luminia and a thicker media, which together lead to vascular hyperreactivity.\(^3\) Hence, a normal vascular resistance in such a structurally altered vascular bed can only be maintained when vascular smooth muscle activity is subnormal, for inevitable geometric reasons.\(^4\)

Only gradually these structural cardiovascular changes regress upon pressure lowering, and complete normalization of the design occurs first after a few weeks.\(^5\) Therefore, the rapid pressure normalization upon renal artery declipping must have a complex background and evidently involves a state of functional hypotension. The present experiments on normotensive rats, with perfusion of acutely declipped RHR kidneys, show that a major (though not necessarily the only) cause of this functional hypoten-

sion is a release of depressor substances from the declipped kidney, thus confirming the findings of Muirhead et al.\(^4\)\(^5\)

References

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