

Factors influencing acute ischaemia-induced renal hypertension in rats

Giorgio Recordati, Federica Zorzoli, Olivia Pontara, Lucia Turolo and Alberto Zanchetti

Objective To verify if the acute hypertension that occurs after reversal of complete renal ischaemia is related to the duration of ischaemia, is different in one-kidney (1K) and two-kidney (2K) rats, and is prevented by angiotensin receptor blockade.

Methods Four groups of Sprague–Dawley rats anaesthetized with pentobarbitone were studied before, during and after a reversible, complete renal ischaemia achieved by functional right nephrectomy.

Results In 1K rats (group 1, $n = 21$), reopening of right renal hilum after functional right nephrectomy of 180, 60 and 30 min was followed by peak increases in systolic blood pressure of 76.0 ± 10.1 mmHg, 36.5 ± 10.0 mmHg and 18.4 ± 4.4 mmHg, respectively (mean \pm SEM). In 2K rats (group 2, $n = 21$), functional right nephrectomy of 180, 60 and 30 min was followed by smaller increases in blood pressure of 49.8 ± 7.6 mmHg, 5.9 ± 3.3 mmHg and 8.3 ± 2.1 mmHg, respectively. Plasma renin activity was directly related to the duration of functional right nephrectomy, and was greater in 1K rats. In group 3, irbesartan administered to 1K rats ($n = 8$) during functional right nephrectomy almost completely prevented the development of hypertension upon reopening. In group 4, labetalol injected intravenously in 1K rats ($n = 3$) did not prevent the blood pressure surge at reopening (49.2 ± 8.5 mmHg).

Conclusions An experimental acute renal hypertension

Introduction

In a recent study of the influence of nephrectomy on efferent renal nerve activity, we showed that, in rats with a single kidney, the reopening of the renal hilum after 3 h of complete renal ischaemia achieved by functional right nephrectomy (FRN) was followed by a prolonged hypertensive and tachycardic episode [1]. Because this hypertensive crisis could be prevented and reversed by intravenous (i.v.) infusion of the angiotensin-converting enzyme inhibitor, captopril, it was suggested that it was caused by the acute release of renin and consequent generation of endogenous angiotensin II (Ang II) [1].

Since the first description of the acute hypertensive

may be elicited both in 1K and in 2K rats and for functional right nephrectomy of 30, 60 and 180 min duration. The increase in blood pressure is proportional to the duration of functional right nephrectomy and greater in 1K than in 2K rats. The experimental acute renal hypertension is due to acute release of renin and generation of endogenous angiotensin II, and is specifically prevented by the angiotensin II type 1 receptor blocker, irbesartan, but not by labetalol. *J Hypertens* 20:2453–2463 © 2002 Lippincott Williams & Wilkins.

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Centro di Fisiologia Clinica ed Ipertensione, Università di Milano, Ospedale Maggiore Milano, Italy.

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Correspondence and requests for reprints to Professor G. Recordati, Centro Fisiologia Clinica ed Ipertensione, Università di Milano, Via F. Sforza 35, 20122, Milano, Italy.
Tel: +39 02 5503 3528; fax: +39 02 503 20480;
e-mail: Giorgio.Recordati@unimi.it

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effects of i.v. injection of renal extracts by Tigerstedt and Bergman [2], the acute response to the reopening of a renal hilum after complete renal ischaemia was the first demonstration of an acute hypertensive effect attributable to endogenous formation of angiotensin II. This response, however, required further characterization. To this end, the present study was undertaken with the following objectives.

First, FRNs of different duration were performed to verify if blood pressure increases and renin release were dependent on the duration of the previous ischaemia and if the increments in blood pressure were related to different magnitudes of renin release. Secondly, because the absence or presence of a contralateral kidney

is known to influence the size and mechanisms of the hypertensive response to occlusion of the renal artery in Goldblatt hypertension [3,4], the blood pressure and renin responses to FRN in rats with and without a preserved contralateral kidney were compared. Thirdly, we investigated whether blockade of the hypertensive outburst could also be achieved by administering a more specific agent, such as the angiotensin II type 1 receptor antagonist, irbesartan, because angiotensin-converting enzyme inhibitors such as captopril are known to block not only the generation of Ang II but also the degradation of vasodilator peptides, and the latter may also be released into the blood stream at the reopening of the renal hilum. Fourthly, in order to verify whether the blood pressure surge was specifically dependent on the activation of the renin-angiotensin system, the effects of irbesartan were compared with those of labetalol, a vasodilator drug acting on α - and β -adrenergic receptors.

In analogy with the nomenclature in current use for the Goldblatt model of hypertension [5], each group of rats that we studied was identified by means of four abbreviations representing, respectively: the number of kidneys remaining in the experimental animal (2K, 1K), the number of renal hila around which a snare was twisted (2S, 1S), the duration in minutes of the total renal ischaemia that was induced (180, 60 or 30), and the identity of the drug injected i.v. before the reopening of the right renal hilum (irbesartan: irbs; labetalol: labt).

Methods

Animal preparation

Fifty-three male Sprague-Dawley rats weighing 200–300 g (Charles River Italia, Calco, Lecco, Italy) were anaesthetized by intraperitoneal injection of sodium pentobarbitone (5 mg/100 g) and maintained at 37°C on a heated operating table. Polyethylene catheters were inserted into: (1) the trachea, to facilitate spontaneous breathing; (2) the right external jugular vein, for continuous infusion of sodium pentobarbitone diluted in 0.85% saline solution at 20 μ l/min (PE-50); (3) the left external jugular vein, for supplementary doses of the anaesthetic and for ad-hoc administration of irbesartan or labetalol (PE-10); (4) the left femoral artery, for monitoring of arterial blood pressure; and (5) the left femoral vein, for withdrawal of blood for measurement of plasma renin activity (PRA). The rats were then divided into four groups: in groups 1 (1K, $n = 21$), 3 (1K-irbs, $n = 8$) and 4 (1K-labt, $n = 3$), all the rats underwent excision of the left kidney (left nephrectomy) by a retroperitoneal approach; in group 2 (2K, $n = 21$), both kidneys were left intact. Groups 1 and 2 were then each subdivided into three groups (1K-180, $n = 5$; 1K-60, $n = 7$; 1K-30, $n = 9$; 2K-180, $n = 5$; 2K-60, $n = 7$; 2K-30, $n = 9$) and group 3 into two groups (1K-

60-irbs, $n = 5$; 1K-180-irbs, $n = 3$). In all rats, FRN (see below) involved ligation of the right renal hilum by snares, which for sake of simplicity was indicated by the abbreviation, 1S. The right kidney and its nerve supply were retroperitoneally exposed and a polyethylene catheter (PE-50 or PE-10) was inserted into the right ureter close to the renal pelvis to collect urine. The visible nerves on both sides of the renal artery were cut.

Functional right nephrectomy

In all rats, thin threads were placed loosely around the right renal artery (distal to the side branch to the adrenal) and the right renal vein, with the aid of a dissecting microscope. These snares could then be tied in close succession (artery first, then vein) in order to isolate the right kidney from the circulation and to produce a complete renal ischaemia while maintaining a normal circulatory function to the adrenal gland. After 180 min (groups 1K-180, 2K-180, 1K-180-irbs, 1K-180-labt), 60 min (groups 1K-60, 2K-60, 1K-60-irbs) and 30 min (groups 1K-30 and 2K-30), the ligatures were loosened, in reverse order with respect to closure, to re-establish blood flow to and from the right kidney and urine output from that side (reopening). At the beginning of the FRN, the continuous infusion of saline was halved to 10 μ l/min. All the experiments were started between 1030 and 1130 h.

Experimental procedure

Arterial blood pressure, heart rate, rate of breathing and rectal temperature were monitored continuously for 1 h before FRN, during the period of FRN, and for 1 h after the termination of FRN. The time of the first measurement (60 min before FRN) was designated time 0. Subsequent measurements were taken at 15 min intervals and lasted 2–3 min each; during the tying of the renal hilum and its reopening, consecutive measurements were taken for periods of 6–10 min and 10–18 min, respectively.

Haematocrit and plasma renin activity

Microhaematocrit was measured at the beginning (time 0) of the experimental procedure, with two samples withdrawn in close sequence and subsequently averaged. In all groups after the end of the experimental procedure, 2 ml of blood were withdrawn from the femoral vein catheter and, on occasions, from the femoral artery, to measure PRA. PRA was determined by incubating plasma samples at 37°C for 1 h and using a commercial radioimmunoassay kit to measure the angiotensin I generated [6], expressed as ng/ml per h. Four measurements of PRA were made in each experimental group, except for group 2K1S-180 (five measurements) and groups 2K1S-30, 1K1S-180-irbs and 1K1S-180-labt (three measurements).

Urine flow rate, osmolality and Na⁺ and K⁺ concentrations

Urine from the experimental right kidney was collected during 1 h periods before FRN (sample 1), and from reopening of the renal hilum to the end of the experiment (sample 2). Urine osmolality was measured with an Osmette A instrument (Precision System Inc., Sudbury, Massachusetts, USA) and urine Na⁺ and K⁺ concentrations with a Nova Biomedical CRT 1 AD (Nova Biomedical, Waltham, Massachusetts, USA).

Laboratory equipment and data analysis

Blood pressure (Gould P23D pressure transducer; Gould-Statham Instrument, Hato Rey, Puerto Rico, West Indies) and electrocardiogram (ECG; Grass P511 preamplifier; Astro-Med, Inc., Rhode Island, USA) were monitored on an oscilloscope (Tektronix 5115; Tektronix Inc., Beaverton, Oregon, USA), stored on a magnetic tape (Racall 4; Racal-Thermionic, Hythe, Southampton, UK) and recorded on a Bryans 40 000 ultraviolet polygraph recorder (Bryans Southern Instruments, Mitcham, Surrey, UK). Heart rate was measured from the electrocardiogram (ECG) with an instantaneous frequency time-meter (Ortec 4672, Oak Ridge, Tennessee, USA). Rate of breathing and temperature were measured with thermoprobes connected to a digital meter (Marazza, Hardware & Software, Monza, Milan, Italy). Blood pressure, ECG, heart rate and rate of breathing were also fed into an analog-to-digital converter connected to a digital acquisition board (National Instruments) and driven by a computer program (Grass PolyView, Astro-Med, Inc.). Data acquisition was obtained at 500 Hz with a Pentium II PC. Each period of 2–3 min was then subdivided into 20–30 s periods, and a single mean calculated for the 2–3 min period.

Statistical analysis

Values are expressed as means \pm SEM. Differences in recorded variables between groups were compared by analysis of variance (single factor or two factors with replications). Subsequently, the recorded variables could be compared within each group with the same variables at time 0, the values at each hour could be compared with those at the preceding hour, and the values after the test stimulus could be compared with those before the stimulus (paired Student's *t*-test, Bonferroni correction for multiple testing). Variables were also compared between groups by unpaired Student's *t*-test. Statistical significance was defined as $P < 0.05$, $P < 0.01$ or $P < 0.001$. The correlation between PRA and changes in systolic blood pressure (SBP) was calculated using linear regression analysis.

Results

Table 1 shows the average peak SBPs, and the magnitude of the changes, at reopening of the renal hilum, compared with values before FRN (time 60 min) for each group of rats; changes within and

Table 1 Average reference systolic blood pressure (SBP) before functional right nephrectomy (FRN) and peak SBP, and magnitude of change after reopening for all groups

Group	SBP before FRN (mmHg)			SBP peak after reopening (mmHg)			SBP change (mmHg)		
	30 min	60 min	180 min	30 min	60 min	180 min	30 min	60 min	180 min
1: 1K	120.7 \pm 2.8	118.9 \pm 4.2	115.1 \pm 4.1	139.1 \pm 5.7**	155.3 \pm 10.6*	191.1 \pm 7.3**	18.4 \pm 4.4	36.5 \pm 10.0†	76.0 \pm 10.1‡
2: 2K	114.1 \pm 3.9	112.1 \pm 5.8	120.0 \pm 3.6	122.4 \pm 4.3**	118.0 \pm 4.0	169.7 \pm 6.9**	8.3 \pm 2.1	5.9 \pm 3.3	49.8 \pm 7.6†††
3: 1K:irbs		112.5 \pm 3.5	118.1 \pm 5.3		106.6 \pm 5.0	123.4 \pm 13.6		-5.9 \pm 4.1††	5.3 \pm 10.7††
4: 1K:labt			119.4 \pm 6.8			168.6 \pm 15.3*			49.2 \pm 8.5†

Values are mean \pm SEM. irbs, irbesartan; labt, labetalol. Within groups comparisons (paired *t*-test for peak compared with before FRN); * $P < 0.05$, ** $P < 0.01$. Between groups (unpaired *t*-test); † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$ for 180 min compared with 60 min; † $P < 0.05$, †† $P < 0.01$ for 1K compared with 2K, 1K compared with 1K:irbs, and 1K:irbs compared with 1K:labt.

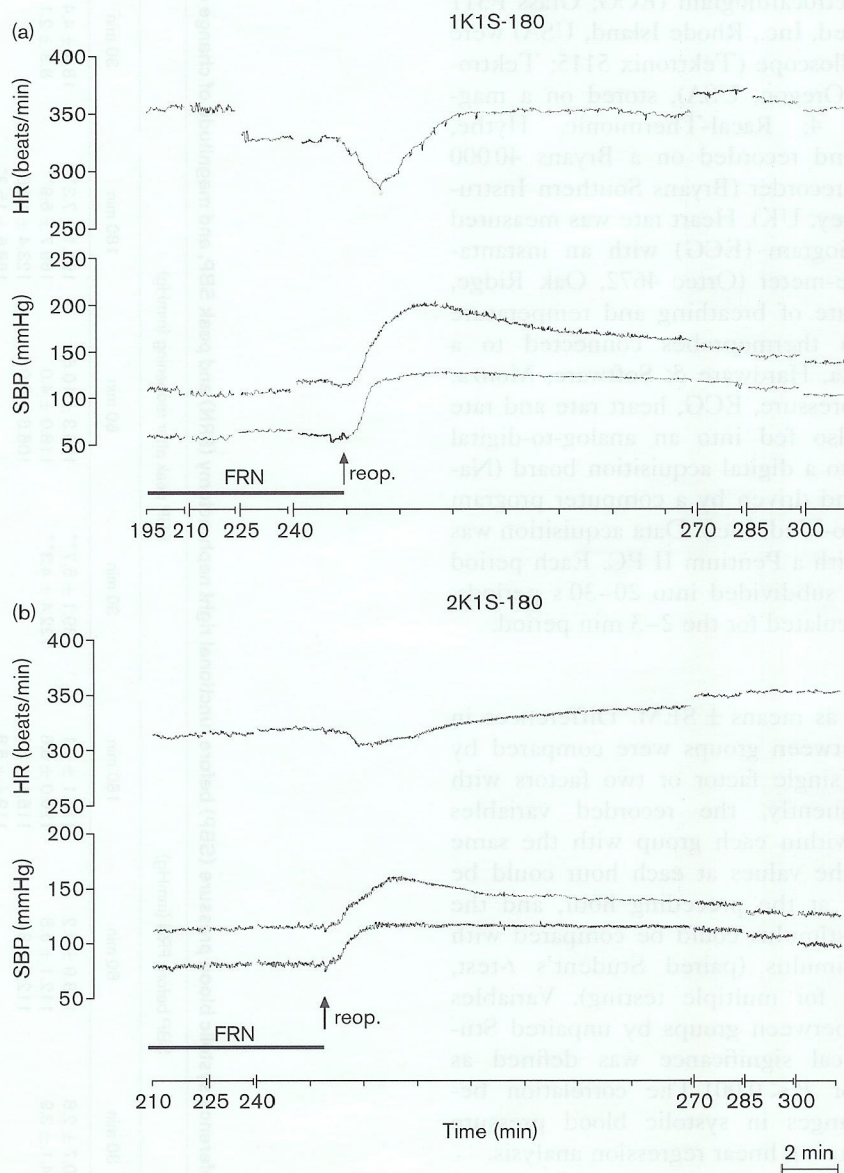
between groups may also be compared. Within each group of animals (1K or 2K), 180 min of FRN consistently induced significantly greater increases in SBP than did 60 and 30 min of FRN; in addition, for the same duration of ischaemia, 1K rats had greater average increases in blood pressure (Table 1). This difference achieved statistical significance for 60 min FRN (Table 1), and approached significance for 180 min FRN ($P = 0.07$). When changes in SBP on reopening were compared with reference blood pressure immediately

before reopening, the difference between the 1K1S-180 and 2K1S-180 groups was significantly different ($P < 0.01$).

Functional right nephrectomy of 180 min in groups 1K1S-180 and 2K1S-180

Figure 1 shows blood pressure and heart rate changes occurring at the reopening of the renal hilum for a representative experiment in groups 1K1S-180 and 2K1S-180. The reopening of the renal hilum after 3 h

Fig. 1



Haemodynamic effects of the reopening of the right renal hilum in representative experiments in groups 1K1S-180 (a) and 2K1S-180 (b). Time base: the numbers below the abscissa indicate the starting time of the recording period with respect to the beginning of the experimental procedure at time 0; there was continuous recording from 240 min to 255 min in (a) and from 240 min to 256 min in (b). The dark bar above the abscissa indicates the last 47 min (a) and 32 min (b) of the period of functional right nephrectomy (FRN). The upward arrow before reopening of the right renal hilum (reop.) indicates the timing at which the renal artery was untied. HR, heart rate; SBP, systolic blood pressure.

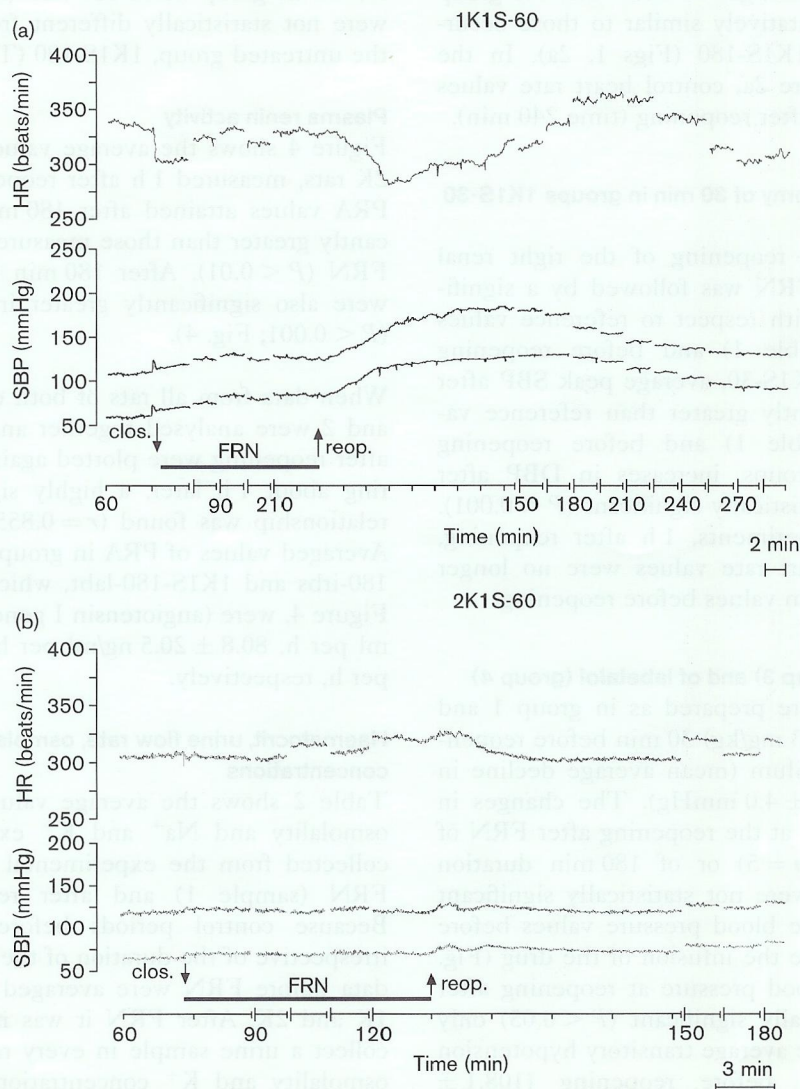
of FRN elicited the most consistent increases in blood pressure in both groups (Table 1). SBP peaks after reopening were significantly different both from reference values immediately before FRN (Table 1) and from values immediately preceding reopening ($P < 0.001$) for both 1K1S-180 and 2K1S-180 rats. Similar statistically significant changes were observed in diastolic blood pressure (DBP; $P < 0.01$). At the end of the experiments, 1 h after reopening, SBP and DBP were still significantly greater than preopening values in both groups (time 300 min in Figs 1 and 2; $P < 0.01$). The rapid increase in blood pressure after the reversal of renal ischaemia was accompanied by a bradycardia of short duration (Fig. 1). However, heart rate started to

recover towards and beyond preopening values before the peak increase in blood pressure was reached (Fig. 1). During the first 1 h after the reopening, heart rate remained more rapid than before reopening, paralleling the persistence of increased blood pressure (Fig. 1), and at 30 min after reopening it was statistically significantly greater than the values before reopening in both groups ($P < 0.05$).

Functional right nephrectomy of 60 min in groups 1K1S-60 and 2K1S-60

Figure 2 shows blood pressure and heart rate changes for a representative experiment in each group. The average SBP peak after reopening for group 1K1S-60

Fig. 2



Haemodynamic effects of closure (clos.) of the right renal hilum for a 60-min period and its reopening (reop.) in representative experiments in groups 1K1S-60 (a) and 2K1S-60 (b). In (a), recordings of heart rate (HR) and systolic blood pressure (SBP) are shown for 2 h and 45 min, and continuous recording from time 120 min to 140 min. FRN, functional right nephrectomy.

(Table 1) was statistically significant with respect to both blood pressure reference values before FRN (Table 1) and the control SBP values immediately before reopening ($P < 0.05$). In group 2K1S-60, the average SBP peak after reopening was not statistically different from blood pressure values before FRN (Table 1), whereas it was significant with respect to blood pressure values before reopening ($P < 0.01$).

One hour after reopening, average SBP and DBP were still significantly greater than control values in group 1K1S-60 only (time 180 min in Fig. 2a; $P < 0.05$). In the experiment shown in Figure 2a, the haemodynamic variables were followed for 2 h and 45 min after reopening. In this example, blood pressure had returned spontaneously to control values almost 2 h after reopening. Although average changes in heart rate were not statistically significant, changes in heart rate for group 1K1S-60 rats were qualitatively similar to those occurring in rats in group 1K1S-180 (Figs 1, 2a). In the example shown in Figure 2a, control heart rate values were resumed only 2 h after reopening (time 240 min).

Functional right nephrectomy of 30 min in groups 1K1S-30 and 2K1S-30

In group 1K1S-30, the reopening of the right renal hilum after 30 min of FRN was followed by a significant increase in SBP with respect to reference values both before FRN (Table 1) and before reopening ($P < 0.001$). In group 2K1S-30, average peak SBP after reopening was significantly greater than reference values before FRN (Table 1) and before reopening ($P < 0.001$). In both groups, increases in DBP after reopening were also statistically significant ($P < 0.001$). At the end of the experiments, 1 h after reopening, blood pressure and heart rate values were no longer statistically different from values before reopening.

Effects of irbesartan (group 3) and of labetalol (group 4)

In group 3, the rats were prepared as in group 1 and received irbesartan i.v. (3 mg/kg) 30 min before reopening of the right renal hilum (mean average decline in blood pressure of 15.6 ± 4.0 mmHg). The changes in blood pressure observed at the reopening after FRN of 60 min (1K1S-60-irbs, $n = 5$) or of 180 min duration (1K1S-180-irbs, $n = 3$) were not statistically significant compared with reference blood pressure values before FRN (Table 1) or before the infusion of the drug (Fig. 3a). The changes in blood pressure at reopening after irbesartan were statistically significant ($P < 0.05$) only when compared with the average transitory hypotension induced by irbesartan before reopening (108.1 ± 14.6 mmHg). Average changes in blood pressure at reopening after irbesartan were consistently and significantly less than those induced by a similar period of FRN in the untreated group 1 (Table 1).

In group 4, the rats were prepared as in groups 1 and 3 and received labetalol i.v. (5.5 mg/kg) as a slow bolus i.v. injection, 30 min before reopening of the right renal hilum (mean average maximum decline in blood pressure of 32.7 ± 4.0 mmHg). When compared with average blood pressure values before FRN (Table 1) or before drug injection, or with the average blood pressure values immediately before reopening (105.0 ± 6.8 mmHg), the increments in blood pressure after reopening of the renal hilum in the 1K1S-180-labt rats were consistent and statistically significant (Fig. 3b, Table 1). When the average magnitude of changes in blood pressure after the reopening was calculated with respect to blood pressures immediately before reopening, the average change amounted to 63.7 ± 8.7 mmHg. The average SBP changes observed after reopening were statistically significantly greater than those observed in group 1K1S-180-irbs ($P < 0.05$; Fig. 3a) and were not statistically different from those observed in the untreated group, 1K1S-180 (Table 1, Figs 1a, 3b).

Plasma renin activity

Figure 4 shows the average values of PRA for 1K and 2K rats, measured 1 h after reopening. In both groups, PRA values attained after 180 min FRN were significantly greater than those measured after 60 and 30 min FRN ($P < 0.01$). After 180 min of FRN, PRA values were also significantly greater in 1K than in 2K rats ($P < 0.001$; Fig. 4).

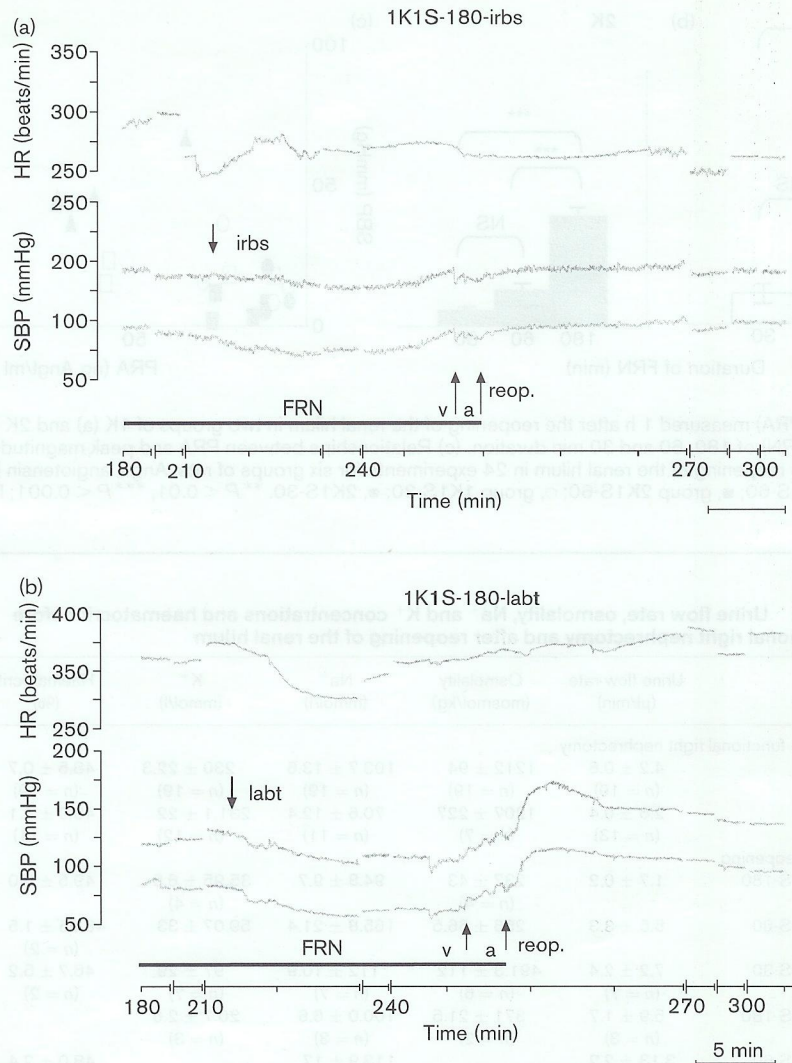
When data from all rats of both experimental groups 1 and 2 were analysed together and peak SBP increases after reopening were plotted against PRA values occurring about 1 h later, a highly significant direct linear relationship was found ($r = 0.855$, $P < 0.001$; Fig. 4c). Averaged values of PRA in groups 1K1S-60-irbs, 1K1S-180-irbs and 1K1S-180-labt, which are not included in Figure 4, were (angiotensin I generated) 86.0 ± 24.8 ng/ml per h, 80.8 ± 20.5 ng/ml per h and 74.6 ± 1.9 ng/ml per h, respectively.

Haematocrit, urine flow rate, osmolality, and Na^+ and K^+ concentrations

Table 2 shows the average values of urine flow rate, osmolality and Na^+ and K^+ excretion for the urine collected from the experimental (right) kidney before FRN (sample 1) and after reopening (sample 2). Because control periods before FRN were similar irrespective of the duration of the following FRN, urine data before FRN were averaged separately for groups 1K and 2K. After FRN it was not always possible to collect a urine sample in every rat. Nonetheless, urine osmolality and K^+ concentration showed a clear decrease in both groups, with a trend toward a smaller decrease as the period of FRN decreased.

At the beginning of the experimental procedure, the

Fig. 3



Haemodynamic effects of reopening (reop.) of the right renal hilum, (a) after a bolus injection of irbesartan (irbs) i.v., and (b) after a slow bolus infusion of labetalol (labt) in 1K1S-180 rats. The bar above the abscissa indicates the last 68 min (a) and 67 min (b) of the period of functional right nephrectomy (FRN). The two upward arrows marked by letters 'v' and 'a' at the end of the period of FRN indicate the separate and sequential reopening of the right renal vein and artery. HR, heart rate; SBP, systolic blood pressure.

haematocrit was $48.5 \pm 0.7\%$ in group 1K ($n = 19$) and $48.0 \pm 1.1\%$ in group 2K ($n = 11$; Table 2). Haematocrit values at the end of the experiment (Table 2) revealed that changes in haematocrit that occurred during the experiment were slight, unpredictable and apparently unrelated to the duration of FRN (Table 2).

Discussion

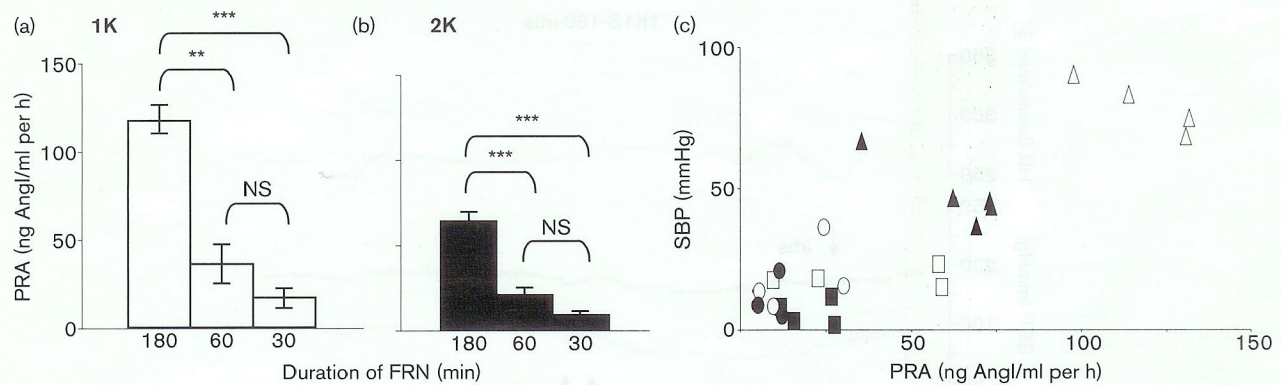
Effects of duration of renal ischaemia

The results of our experiments show that, in both 1K and 2K rats, the reperfusion of a kidney after a period of complete renal ischaemia is followed by a hypertensive surge. This experimental acute renal hypertension was highly reproducible for periods of 180 min

of FRN in both 1K and in 2K rats, and more variable for shorter periods of FRN, of 60 and 30 min. The extent of the blood pressure response in groups 1K and 2K was found to be in direct relation to the duration of the FRN and to the PRA measured 1 h after the reopening of the renal hilum (Table 1, Fig. 4c).

The duration of renal ischaemia seems, therefore, to be the determinant of both the increase in blood pressure and the formation of Ang II. This observation may be explained by the assumption that hyperangiotensinaemia is the result of ischaemic and post-ischaemic tubular and cortical injury [7-9]. Increasing the duration of FRN will increase the extent of renal damage,

Fig. 4



Average plasma renin activity (PRA) measured 1 h after the reopening of the renal hilum in two groups of 1K (a) and 2K (b) rats, for periods of functional right nephrectomy (FRN) of 180, 60 and 30 min duration. (c) Relationships between PRA and peak magnitude of change in systolic blood pressure (SBP) observed at the reopening of the renal hilum in 24 experiments for six groups of rats. Ang I, angiotensin I. Δ , group 1K1S-180; \blacktriangle , group 2K1S-180; \square , group 1K1S-60; \blacksquare , group 2K1S-60; \circ , group 1K1S-30; \bullet , 2K1S-30. $**P < 0.01$, $***P < 0.001$; NS, not statistically significant.

Table 2 Urine flow rate, osmolality, Na^+ and K^+ concentrations and haematocrit before functional right nephrectomy and after reopening of the renal hilum

Group	Urine flow rate ($\mu\text{l}/\text{min}$)	Osmolality (mosmol/kg)	Na^+ (mmol/l)	K^+ (mmol/l)	Haematocrit (%)
Before functional right nephrectomy					
1K	4.2 ± 0.6 (n = 19)	1212 ± 94 (n = 19)	103.7 ± 13.6 (n = 19)	230 ± 22.3 (n = 19)	48.5 ± 0.7 (n = 19)
2K	2.6 ± 0.4 (n = 13)	1207 ± 227 (n = 7)	70.6 ± 12.4 (n = 11)	231.1 ± 22 (n = 12)	48.0 ± 1.1 (n = 11)
After reopening					
1K1S-180	1.7 ± 0.2	237 ± 43 (n = 4)	94.8 ± 9.7	35.95 ± 6.5 (n = 4)	49.5 ± 0.0
1K1S-60	5.5 ± 3.3	256 ± 66.5	165.8 ± 21.4	59.07 ± 33	48.25 ± 1.5 (n = 2)
1K1S-30	7.2 ± 2.4 (n = 7)	491.3 ± 112 (n = 6)	112 ± 10.9 (n = 7)	97 ± 29 (n = 7)	46.7 ± 5.2 (n = 2)
2K1S-180	5.9 ± 1.7 (n = 3)	371 ± 21.5 (n = 2)	100.0 ± 6.6 (n = 3)	20.7 ± 2.6 (n = 3)	
2K1S-60	3.13 ± 2.2 (n = 3)		113.9 ± 17 (n = 2)		48.0 ± 2.4 (n = 3)
2K1S-30	3.6 ± 2.0 (n = 2)	760 ± 98 (n = 2)	95.5 ± 14.5 (n = 2)	265.0 ± 115.1 (n = 2)	49.7 ± 1.8 (n = 8)

Values are mean \pm SEM. Where number of observations is not specified, n = 5.

the production of Ang II formation, and the subsequent increase in blood pressure at the reopening of the renal hilum.

Comparison between 1K and 2K rats

For any period of ischaemia, the blood pressure responses at reopening and PRA were greater in 1K rats than in 2K rats. This observation raises the possibility that the contralateral intact left kidney might have filtered and inactivated renin [10], or produced vasodilating protective substances such as medullipin [11–13], nitric oxide, prostaglandins and lipoxigenase [14]. The finding that the haematocrit was not greater in 1K rats either before or after FRN makes it unlikely that a major increment in blood volume occurring during the

period of FRN in 1K rats had contributed to the greater pressor response of these animals.

Blockade of blood pressure response to reopening

In a previous study, we have also demonstrated that the acute hypertension that follows the release of a FRN of 180 min duration in 1K rats is either completely prevented by captopril infused before the reopening of the renal hilum, or reversed by captopril infused i.v. a few minutes after the development of the hypertension [1]. In the present series of experiments, it has been shown that the i.v. infusion of the angiotensin II type 1 receptor blocker, irbesartan, prevented the development of the hypertension in 1K1S-60-irbs and 1K1S-180-irbs rats. In contrast, labetalol, an α - and β -

adrenergic blocker, at the dosage we used to reproduce a hypotensive effect similar to that induced by irbesartan, did not prevent the release of renin and the hypertensive surge at the reopening of the renal hilum. It is interesting that labetalol, although possessing β -blocking properties, did not affect the release of renin on reopening, probably because total renal ischaemia prevented the intrarenal β -blocking action of labetalol during FRN. It can be concluded, therefore, that only those drugs that specifically block the peripheral actions of the renin-angiotensin system seem to be able to prevent the increase in blood pressure at the reopening of the ischaemic kidney, and that the experimental acute renal hypertension is almost certainly due to the acute release of renin and to an acute action of endogenous Ang II on target organs [1].

To our knowledge, the first evidence of an acute hypertensive effect of endogenous Ang II upon the removal of a clamp positioned on the renal artery of a 2K animal (dog) after 60 min of complete renal ischaemia was given by Hosie *et al.* [15]. This finding was not commented on or further investigated thereafter. Hence, the present paper may be considered to be the first detailed description of an experimental model of acute renovascular hypertension produced by the reopening of a renal hilum after complete renal ischaemia.

Heart rate response

One of the main characteristics of experimental acute renal hypertension is that it is accompanied by very brief periods of bradycardia at the beginning of the increase in blood pressure, only when this increase is marked and fast (Fig. 1) [1]. Conversely, when blood pressure increases slowly, as occurs at the release of a 30 min FRN, no bradycardia occurs and a modest increase in heart rate parallels the increase in blood pressure. This tachycardic effect may be dependent on a central [16] or peripheral action of Ang II on the visceral nervous system or directly on the heart, with the final effect of resetting the baroreceptor reflex to greater pressure [17–20].

Unless Ang II is injected intrarterially [16], the cardiovascular effects of exogenously injected Ang II are very short lasting and never accompanied by tachycardia [19]. This observation raises the basic question of whether, as has already been demonstrated for acetylcholine and hypothesized for catecholamines [21,22], endogenous Ang II may act as neuromodulator and at the target-organ site in a manner different from the action of exogenously infused Ang II [18,19,23–26]. The more prolonged action of endogenous Ang II may also be simply explained by taking into account the release of renin, the half-life of which is much longer than that of Ang II itself.

Role of renal innervation

In the experimental acute renal hypertension, the experimental kidney was denervated, hence a possible contribution of renal sensory innervation can be excluded [27–31]. This confirms that experimental acute renal hypertension is also quite different, both from the model of the 'renal pressor reflex' based on a neural reflex of renal origin elicited by a partial constriction of the renal artery, in rats with baroreceptor denervation and renin-angiotensin blockade [32,33], and from the model of neurogenic hypertension caused by intrarenal injection of phenol and by 5/6 nephrectomy [34,35].

In contrast, the role of efferent sympathetic nerves remains to be clarified, because a marked, although short-lasting, excitation of the efferent sympathetic nerve to the experimental kidney coincident with the peak increase in blood pressure has been recorded at the reopening of the renal hilum in four of five 1K1S-180 rats [1,36,37]. This increase in renal sympathetic nerve activity is in line with the recently demonstrated central action of Ang II in inhibiting the neuronal isoform of nitric oxide synthase and interleukin-1 β in the posterior hypothalamic and paraventricular nuclei and locus ceruleus [38,39].

Experimental acute renal hypertension and the Goldblatt model of experimental hypertension

The work by Liard *et al.* [40] is one of the very few studies that aimed to measure acute changes in blood pressure in one kidney-one clip (1K1C) Goldblatt hypertension. These authors demonstrated that a consistent increase in blood pressure and release of renin occurred immediately after clip insertion in baroreceptor and sinoaortic-denervated 1K1C dogs [40]. In contrast, prompt decline of the blood pressure values towards control values after clip removal has been well documented in both 1K and 2K animals [41–43]. Furthermore, Goldblatt hypertension and experimental acute renal hypertension share one feature – namely, that the increase in blood pressure in 1K1C and in 1K1S animals is faster and larger than that in 2K1C and 2K1S animals, confirming the protective role of the contralateral kidney in both models [3,4,44–46].

Experimental acute renal hypertension and acute renal failure

In our approach, the renal artery and vein were occluded sequentially. At variance with our model, simultaneous and complete occlusion of the renal artery and vein of the experimental kidney for 30–180 min periods has been used to reproduce an acute ischaemic renal failure – the ischaemia-reperfusion model – in mice, rats, rabbits and dogs [7–9,47]. Alterations in urine flow rate, osmolality, and Na⁺ and K⁺ excretion measured after the FRN in our experiments agree with the work on acute renal failure and demonstrate that

tubular damage had occurred [7]. In the rat, cortical damage usually occurs after 60 min of complete ischaemia only, whereas in humans, periods of warm ischaemia up to 75 min are tolerated by transplants without giving rise to significant acute renal failure [7,48].

In conclusion, the demonstration of an acute vasoconstrictor effect of endogenous Ang II may contribute to a better understanding of Goldblatt hypertension, and of those clinical conditions that are characterized by an activation of the renin-angiotensin system, such as hypertensive crises of renal origin [49,50] and hypertension occurring after renal transplantation [51,52].

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