# Effects of a reversible 'nephrectomy' on renal sympathetic activity and blood pressure in the rat: evidence for an acute angiotensin-mediated hypertension

Giorgio Recordati, Federica Zorzoli and Alberto Zanchetti

***Objective*** **To verify whether the normal kidney exerts a supportive, facilitatory action on renal sympathetic nerve activity (RSNA), the effects of unilateral and bilateral nephrectomy on RSNA have been studied.**

***Methods*** **The RSNA, rectal temperature (T), rate of breathing (RB), arterial blood pressure (BP) and heart rate (HR) were continuously recorded in three groups of pentobarbital anaesthetized, spontaneously breathing Sprague–Dawley rats: group 1 ($n = 5$): both kidneys intact; group 2 ($n = 5$): left surgical nephrectomy; group 3 ($n = 5$): left surgical nephrectomy and functional exclusion of the right kidney (functional right nephrectomy, FRN), produced by a tight ligature of the renal hilum which was maintained for 3 h. In a fourth group ($n = 7$), in which nerve activity was not recorded, reopening of the right renal hilum was preceded or followed by intravenous administration of captopril (3 mg/kg).**

***Results*** **In groups 1 and 2 RSNA increased from 22.3 ± 2.1 to 122.9 ± 13.6 and from 26.7 ± 1.2 to 93.2 ± 14.0 impulses/s (mean ± SEM), respectively, without concomitant changes in cardiovascular parameters. In group 3 RSNA decreased from 39.1 ± 3.1 to 13.7 ± 2.6 impulses/s during the 3 h of FRN. In group 3 the reopening of the right renal hilum was followed by a marked increase in BP and HR that was prevented or reversed by intravenous captopril in rats of group 4.**

***Conclusions*** **The decrease in RSNA observed in rats during bilateral nephrectomy, in contrast to the increase observed in rats with one or both kidneys intact, suggests that the kidney as a whole exerts a supportive role on sympathetic nerve activity. The hypertension and tachycardia that follows the reopening of the right kidney hilum appears to be caused by the generation of endogenous angiotensin II; this is the first evidence of an acute angiotensin-mediated renal hypertension. *J Hypertens* 2000, 18:1277–1287 © Lippincott Williams & Wilkins.**

Journal of Hypertension 2000, 18:1277–1287

**Keywords: sympathetic nerve activity, renal nerve activity, nephrectomy, functional nephrectomy, renal sensory innervation, renal chemoreceptors, experimental hypertension, acute renal hypertension, acute angiotensin-mediated hypertension, angiotensin converting enzyme inhibitors**

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**Received** 7 December 1999 **Revised** 19 April 2000
**Accepted** 16 May 2000

## Introduction

Efferent renal sympathetic nerve activity (RSNA) has been studied in several animal models and under a variety of different experimental conditions [1,2]. The kidney is known to be not only the target of this sympathetic activity, but also the origin of neural and non-neural influences that act on the sympathetic nervous system [1–3], the activity of which fluctuates continuously and is commonly lower in conscious animals than in those under anaesthesia [4–6]. Several years ago, Zimmermann *et al.* [7] pointed out the facilitatory actions exerted by angiotensin II on sympathetic activity at central and peripheral levels; the interactions between the renin–angiotensin system and renal sympathetic activity were described by Zanchetti [3] as a true positive feedback mechanism.

A reflex sympathetic activating role has also been postulated for the renal sensory innervation, particularly the renal chemoreceptors [8–10]. Converse *et al.* [11] have shown that in patients with chronic renal failure bilateral nephrectomy is followed by a marked decline in muscle sympathetic nerve activity; they have advanced the hypothesis that renal chemoreceptors may sustain, at least in this diseased state, the afferent limb of a sympathetic excitatory viscerosomatic reflex.

Although acute nephrectomy is a complex intervention, which does not allow a distinction to be made between the role played by renal sensory innervation and the effects of substances originating from the kidney, it is also a simple way to measure the nature and the extent of the influences on RSNA exerted by the kidney as a whole. This information is prerequisite to the investiga-

tion of the role exerted by specific neural and humoral mechanisms. Furthermore, it is necessary to know whether a 'normal' kidney exerts a supportive excitatory role on sympathetic nerve activity similar to that proposed by Converse *et al.* [11] for the failing kidney. This issue could obviously be investigated in anaesthetized animal preparations only.

To answer these questions at least partially, we recorded RSNA continuously for 5 h in rats with both kidneys intact (group 1), after left nephrectomy (group 2) and before, during and after bilateral nephrectomy (group 3), to verify whether the normal kidney of an anaesthetized animal exerts a supportive and facilitatory function on sympathetic nervous system activity.

Although left nephrectomy was carried out by surgical excision, the nephrectomy on the right side was performed by tying the right renal artery, vein and ureter in close succession. We describe this experimental intervention as 'functional right nephrectomy' (FRN). In comparison with a surgical nephrectomy it offers the great advantage of being reversible at least in the first few hours: by untying the ligatures, renal blood flow may be re-established, and renal function recovers completely or partially, depending on the duration of FRN.

An additional advantage of this type of experimental intervention is that it can be applied under precise microscopic control and without any great distortion of the operating field, so that a functional nephrectomy can be carried out during the recording of the nervous activity from either an efferent or an afferent renal nerve bundle, carefully isolated before ligature of the hilum.

The reversibility of the right nephrectomy also allowed investigation, in a fourth group of rats, of the effects of captopril on the haemodynamic response to the reopening of the renal hilum of the functionally excluded right kidney.

## Methods

### Animal preparation

Twenty-two male Sprague–Dawley rats weighing 200–300 g (Charles River Italia, Calco (Lecco), Italy) were anaesthetized by intraperitoneal injection of sodium pentobarbital (5 mg/100 g) and maintained at 37°C on a heated operating table. Polyethylene catheters were inserted into (1) the left external jugular vein for continuous infusion of 0.85% saline solution at 20 µl/min (PE-50) and intermittent sustaining doses of anaesthetic (PE-10); (2) the trachea to facilitate spontaneous breathing; (3) the left femoral artery to monitor arterial blood pressure; and (4) the left femoral vein to withdraw blood for measurement of plasma renin activity (PRA).

The rats were then divided into four groups: in group 1 ($n = 5$) both kidneys were left intact; in groups 2 ($n = 5$), 3 ($n = 5$) and 4 ($n = 7$) all the rats underwent excision of the left kidney (left nephrectomy), by a retroperitoneal approach. Then 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. Constant circulatory and respiratory conditions and levels of anaesthesia were maintained as far as possible.

#### *Functional right nephrectomy*

In the rats of groups 3 and 4, thin threads were loosely placed around the right renal artery (distally to the side branch to the adrenal), the right renal vein and the right ureter, with the aid of a dissecting microscope. These threads could then be tied to isolate the right kidney from the circulation and to produce a complete renal ischaemia while maintaining a normal circulatory function to the adrenal. After 3 h of FRN, these ties could be loosened to re-establish blood flow to and from the right kidney and urine output (reopening, REOP). At the beginning of the FRN the continuous infusion of saline was halved to 10 µl/min.

All the experiments were started between 1030 and 1130 h.

### Recording of nerve activity

In the rats of groups 1, 2 and 3, a renal nerve bundle was dissected from the tissues along the renal artery, cut peripherally and positioned on bipolar hook electrodes for extracellular recordings. The techniques of dissection of a nerve bundle into small filaments and for recording from small multifibres have been described elsewhere [8–10].

The nerve was left undisturbed at least for half an hour before the start of the experiment. For the duration of the experiment the nerve was left untouched and its position on the electrode remained unchanged.

### Plasma renin activity

Plasma renin activity was determined by incubating plasma samples at 37°C for 1 h and measuring the angiotensin I generated using a commercial radioimmunoassay kit. It was expressed as ng AI/ml per h [12].

### Experimental protocol

The experimental protocol was started at time zero and ended at time 5 h, thus efferent RSNA, arterial blood pressure (BP), heart rate (HR), rate of breathing (RB) and rectal temperature (T) were recorded continuously for 5 h. The first recording period (RP) of the variables was designated RP zero (time 0). Subsequent RPs were taken at 15 min intervals and lasted 2–3 min (see

below). Hence 21 RPs were obtained (from RP 0 to RP 20); the tying of the renal hilum and its reopening were continuously recorded for periods of 6–10 min and 10–18 min, respectively. In groups 1, 2 and 3, immediately after the end of the experimental protocol, 1 ml blood was withdrawn from the femoral vein catheter to measure PRA.

### Drugs

The experimental protocol reported above was also followed for the experiments in group 4 rats, in whom nerve activity was not recorded. In three rats, at time 3.5 h (i.e. 2 h and 30 min after the start of FRN and 30 min before REOP) and in four rats a few minutes after REOP, the angiotensin-converting enzyme inhibitor captopril (Bristol-Myers Squibb Company, Princeton, New Jersey, USA) was intravenously injected at a dose of 3 mg/kg to study its effects on the haemodynamic responses to REOP.

In the four experiments in which captopril injection followed REOP, the protocol was extended to 5 h 30 min.

### Laboratory equipment

Nerve activity (Grass P511 preamplifier, Astro-Med, Milan, Italy), arterial blood pressure (Gould P23D pressure transducer, Gould–Stratham Instrument, Hato Rey, Puerto Rico, West Indies) and ECG (Grass P511 preamplifier) were monitored on an oscilloscope (Tektronix 5115, Tektronix, Vimodrone, Italy), recorded for storage on a magnetic tape (Recall 4, RES, Milan, Italy) and recorded on a Bryans 40 000 ultraviolet polygraph recorder (Hi-Tech, Milan, Italy). Heart rate was measured with an instantaneous frequency time-meter (Ortec 4672, Oak Ridge, Tennessee, USA) from the ECG. Rate of breathing and T were measured with thermoprobes connected to a digital meter (Marazza, Hardware & Software, Monza, Milan, Italy). Subsequent analyses of the nerve activity were aided by a digital neural spike analyser [13,14] and by a computer program (Grass PolyView, Astro-Med, Milan, Italy).

### Data analysis

Recorded variables were played back to an analogue–digital converter connected to a digital acquisition board (National Instruments) and driven by a computer program (Grass PolyView). Data acquisition was performed at 5–10 MHz. Each control period of 2–3 min was then subdivided in shorter periods of 10, 20 and 30 s. The mean of each single variable was calculated using the computer program for these shorter periods, and a single mean calculated for the 2–3 min control period.

Changes in RSNA were quantified either in number of impulses/s and in number and peak amplitude of trains of impulses ('bursts'). For the former RSNA was fed into the spike analyser, which rectified every single impulse above a given threshold, counted them on a time base of 1, 2, 5 or 10 s, and gave an analogue output linearly related to the number of impulses. This output was digitally converted and subsequently analysed with the aid of the computer program. To quantify the number and amplitude of the bursts, a simplification of the method described by Malpas and Ninomiya has been applied [4–6]. Renal sympathetic nerve activity was digitally converted and subsequently integrated on a time constant of 20 ms by the PolyView program. Each burst gave rise to a discrete integrated wave, the amplitude and duration of which was correlated with those of the single burst [4–6]. For each experiment an arbitrary threshold was chosen and the number of integrated bursts above the threshold was counted for periods of 10 s, and for 2–3 min, at time 0, time 1 h and time 4 h. The peak and mean amplitude of the integrated waves was calculated by the PolyView program for each 10 s period.

### Statistical analysis

Values were expressed as means ± SEM. Differences in recorded variables between groups were first of all compared by analysis of variance (ANOVA: single factor and two factors with replications). Subsequently within each group the values of the recorded variables were compared with the values of the same variables at time 0 (paired Student's $t$ test, Bonferroni correction for multiple testing) and the values at each hour were compared with the values at the preceding hour (paired Student's $t$ test). Between groups, the values of the recorded variables were compared by unpaired Student's $t$ test. Significance was defined as $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

## Results

The time needed for surgical preparation of the rats, isolation of the nerves and resting period of the nerve on the electrode, that is the time interval from anaesthesia to the beginning of the experimental protocol, was not statistically different between groups 1, 2 and 3 (177 ± 8, 186 ± 10 and 192 ± 15 min, respectively).

The main condition for a positive experiment was that the nerve should have remained in the same position and untouched on the recording electrode from the beginning (time 0) to the end (time 5 h) of the experimental period. In addition no stimulus was applied during the recording period to avoid any influence on the spontaneous RSNA. Particular attention was also paid to keeping the general conditions of the rats constant throughout the recording period. The intravenous infusion of additional doses of anaesthetics, when required, and the hourly withdrawal of blood from the tail of the rat for haematocrit measurement

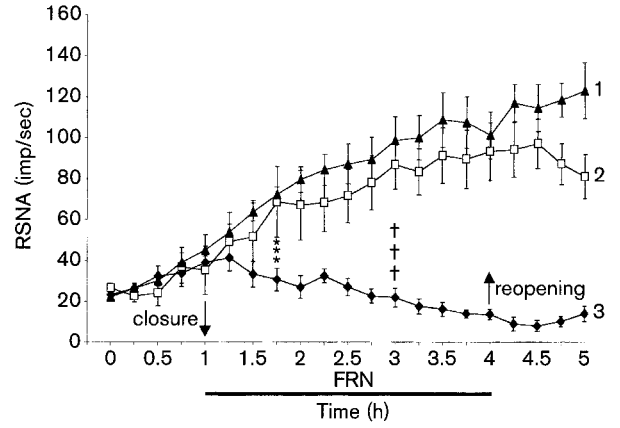
were the only interventions made. No statistically significant differences in T and RB between groups 1, 2 and 3 were observed.

### Two-kidney rats: group 1 ($n = 5$)

The time courses of mean changes in T, RB, HR and BP (Fig. 1) and RSNA (Fig. 2) for groups 1, 2 and 3 have been charted. In group 1, although T, RB, HR and BP did not exhibit any consistent and significant change, the RSNA progressively increased from 22.3 ± 2.1 impulses/s, (time 0, mean ± SEM) to 122.9 ± 13.6 impulses/s at the end of the experiment (time 5 h). This increase, when tested by paired $t$ test at each hour against time 0, was statistically significant from time 1 h (45.1 ± 7.4 impulses/s, $P < 0.05$). When the mean RSNA at each hour was compared with the mean RSNA of the previous hour, it was found that this increase was statistically significant for the first 2 h only ($P < 0.05$), indicating that the mean RSNA reached a plateau after 2.5–3 h from the beginning of the experiment.

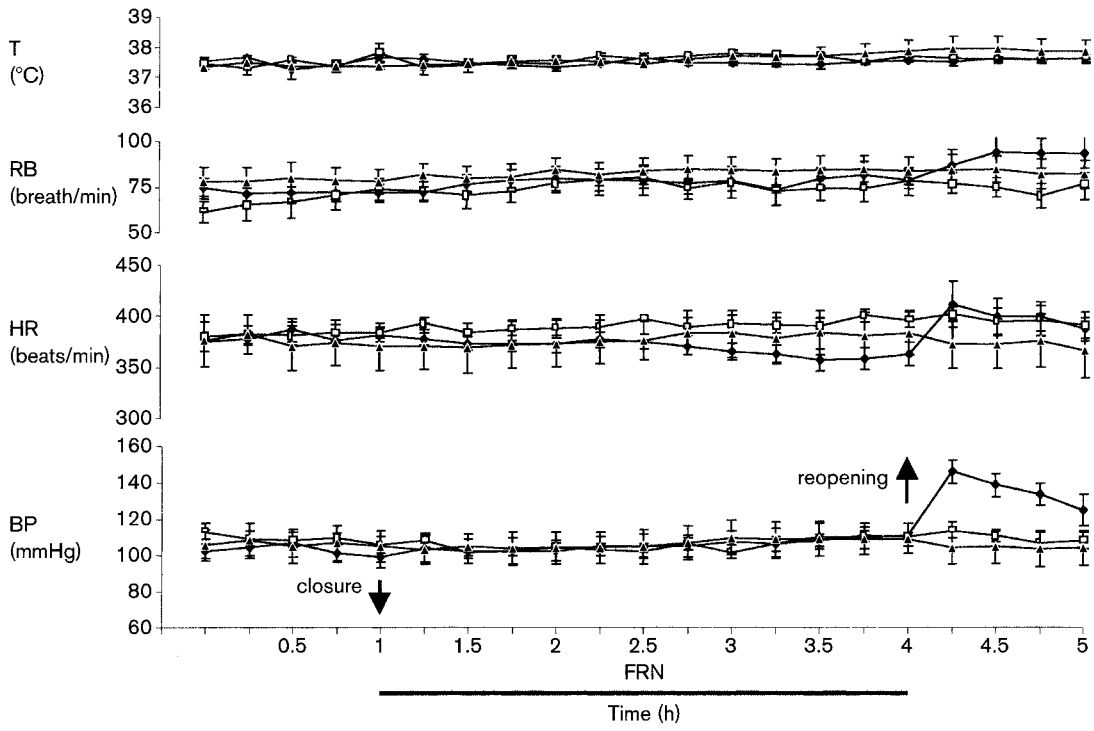
From a quantitative evaluation of the integrated nerve

**Fig. 2**




Time course of mean changes in RSNA for groups 1 (▲) 2 (□) and 3 (◆) during 5 h of continuous recording. Values are means ± SEM. The tick bar marked as functional right nephrectomy (FRN) below the abscissa, which indicates the period and duration of FRN, and the downward ('closure') and upward ('reopening') arrows refer to group 3 only. *** $P < 0.001$ group 3 versus group 1; ††† $P < 0.001$ group 3 versus group 2.

**Fig. 1**




Time course of mean changes in rectal temperature (T), rate of breathing (RB), heart rate (HR) and mean arterial blood pressure (BP) for the rats of groups 1 (▲), 2 (□) and 3 (◆) during 5 h of continuous recording. Values are means ± SEM. The tick bar marked as FRN below the abscissa, which indicates the period and duration of FRN, and the downward ('closure') and upward ('reopening') arrows refer to group 3 only. After reopening, the mean changes in HR and BP are represented at 15 min intervals and therefore the maximum changes in these variables, which occurred between time 4 h and 4.25 h, are not depicted here.

recordings on a time constant of 20 ms it appears that this increase was characterized by an increase in the number of bursts/s (from a mean value of $2.3 \pm 0.1$ bursts/s at time 0 to $5.2 \pm 0.4$ at time 4 h, $P < 0.001$) and by an increase in the peak amplitude of bursts (from a mean peak value of $18.8 \pm 5.8$ V at time 0, to $37.7 \pm 10.4$ V at time 4 h, $P < 0.05$).

The mean haematocrit values for group 1 are given in Table 1. Plasma renin activity measured after the end of the experimental protocol was $4.05 \pm 0.47$ ng AI/ml per h ($n = 4$).

**One-kidney rats: group 2 ($n = 5$)**

The time courses of mean changes in T, RB, HR and BP and the mean changes in RSNA for the five rats in group 2 are also shown in Figures 1 and 2. Again the mean T, RB, HR and BP did not change significantly during the 5 h of recording. Renal sympathetic nerve activity also increased in group 2 rats, but this increase was less uniform, less consistent and 1 h delayed compared with that observed for group 1. When tested by paired $t$ test at each hour against time 0 ($26.7 \pm 1.2$ impulses/s) the increase in RSNA reached statistical significance at 3 h only ($86.7 \pm 11.9$ impulses/s, $P < 0.05$). When the mean level of RSNA at each hour was compared with that of the previous hour, the increase was found significant between the first and second, and second and third hours only. The spontaneous increases in RSNA of group 1 and 2 were not significantly different from one another, however.

As for group 1, the increase in RSNA found in group 2 was characterized by an increase in the number of bursts/s (from $2.3 \pm 0.1$ bursts/s at time 0, to $4.4 \pm 0.4$ bursts/s at time 4 h, $P < 0.01$) and in peak amplitude of the bursts (from $13.4 \pm 1.7$ V at time 0 to $20.1 \pm 2.2$ V at time 4 h, $P < 0.05$).

The mean haematocrit values at each hour for the rats of group 2 were higher than for those of group 1 (Table 1), although they were not statistically different. The mean PRA of group 2 was $7.85 \pm 3.03$ ng AI/ml per h ($n = 4$) and not statistically different from that of group 1.

**Bilateral nephrectomy: group 3 ($n = 5$)**

In the five rats of group 3, the right renal hilum was tied after 1 h of control recording and FRN was maintained for 3 h during which time nerve activity was continuously recorded. The right renal hilum was then reopened and the recording was continued for an additional hour. The data from group 3 rats have been divided into three different periods for analysis: period 1, first hour of control recording; period 2, 3 h of FRN; period 3, REOP of the ischaemic right kidney.

***Periods 1 and 2: one-hour control and FRN***

The time courses of the mean changes in T, RB, HR and mean BP (Fig. 1) and RSNA (Fig. 2) were again illustrated. During the first hour of control recording before FRN, HR and mean BP remained fairly stable (Fig. 1). During FRN, mean HR slowly declined from $381.9 \pm 6.7$ bpm at time 1 h to $364.1 \pm 11.8$ bpm at time 4 h ($P < 0.05$). The mean BP did not show any consistent or significant change during either the first hour of control recording or the 3 h of FRN.

The mean RSNA (Fig. 2) increased during the first hour of control recording before FRN from $23.2 \pm 2.5$ impulses/s (time 0) to $39.1 \pm 3.1$ impulses/s (time 1 h, $P < 0.05$) and the number of bursts increased from $2.4 \pm 0.1$ bursts/s at time 0, to $3.7 \pm 0.3$ bursts/s at time 1 h ($P < 0.05$). During the 3 h of FRN mean RSNA declined slowly and progressively. When tested against the mean level of activity immediately before FRN (time 1 h), this decrease was statistically significant after 1 h of FRN ($P < 0.05$) and more significant after 3 h of FRN ($P < 0.01$; time 4 h, $13.7 \pm 2.6$ impulses/s). When the mean RSNA at each hour was compared with that of the previous hour during FRN, the decrease in RSNA was significant only during the first hour.

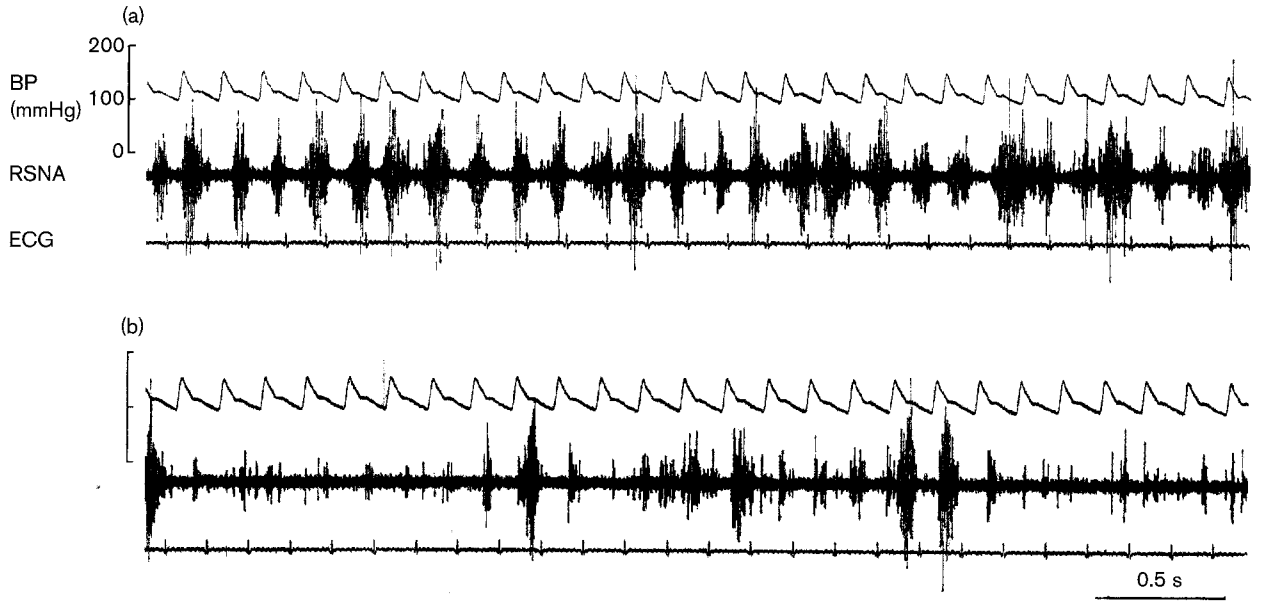
The haematocrit values for group 3 were significantly higher at time 0 and time 1 h compared with group 1 values only and did not change significantly during the experimental period (Table 1). Figure 3 shows the analogue recording of BP, RSNA and ECG immediately before (time 1 h, Fig. 3a) and at the end of the FRN period (time 4 h, Fig. 3b) for one rat from group 3. Activity clearly decreases during FRN. The decrease in RSNA in group 3 was characterized by a clear decrease in the number of bursts/s (from $3.7 \pm 0.3$ at time 1 h to $1.2 \pm 0.4$ at time 4 h, $P < 0.01$) and by a decrease in the peak amplitude of the bursts, although this was not statistically significant (from $3.5 \pm 0.2$ V at time 1 h to $2.7 \pm 0.3$ V at time 4 h).

Table 1 **Haematocrit values (%) for the rats of groups 1, 2 and 3 at each hour**

| | 0 h | 1 h | 2 h | 3 h | 4 h | 5 h |
|---|---|---|---|---|---|---|
| Group 1 | $43.5 \pm 1.3$ | $43.9 \pm 1.3$ | $44.1 \pm 1.4$ | $43.5 \pm 1.7$ | $43.9 \pm 1.6$ | $43.7 \pm 2.3$ |
| Group 2 | $46.4 \pm 1.0$ | $44.6 \pm 0.1$ | $44.6 \pm 0.9$ | $45.1 \pm 0.7$ | $46.0 \pm 0.7$ | $45.7 \pm 0.9$ |
| Group 3 | $48.4 \pm 0.7$* | $47.6 \pm 0.9$* | $47.7 \pm 1.3$ | $47.3 \pm 1.4$ | $48.1 \pm 0.9$ | $47.9 \pm 0.7$ |

Data are means ± SEM. * $P < 0.05$ with respect to group 1.

Fig. 3




Analogue recording of arterial blood pressure (BP), renal sympathetic nerve activity (RSNA) and ECG for one animal from group 3, showing the level of spontaneous activity from the same multifibre preparation at (a) time 1 h, immediately before the start of the functional right nephrectomy (FRN), and (b) the end of the 3-h FRN period, immediately before the reopening at time 4 h.

Thus the RSNA response during the 3-h period of FRN (bilateral nephrectomy) in the rats of group 3 is in contrast to that observed during 5-h experiments in groups 1 and 2 (Fig. 2). Between groups 1 and 3 changes in RSNA in impulses/s became highly statistically different ($P < 0.001$) 45 min after the beginning of FRN (time 1.75 h), whereas between groups 2 and 3 they became highly statistically different ($P < 0.001$) after 2 h of FRN (time 3 h). Differences in the time course of changes for RSNA between groups 1 and 2 rats were never significant.

### *Reopening of the ischaemic kidney*

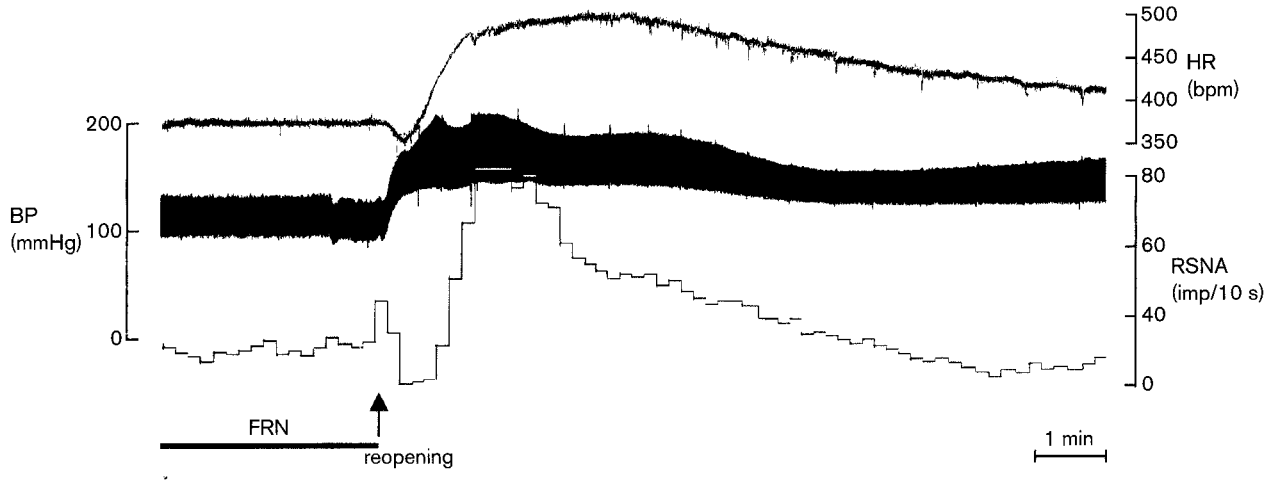
The reopening of the right renal hilum after 3 h of FRN was followed by a marked increase in BP and, after a short period of bradycardia, by a consistent tachycardia (Fig. 1). The effects of the reopening of the ischaemic (and only remaining) right kidney after 3 h of FRN are documented in Figure 4 for a single animal from group 3. The RSNA was slightly excited during the operation of untying the right renal hilum, then it was almost completely silenced at the beginning of the blood pressure rise. The RSNA showed a brief period (3–4 min) of intense excitation at the peak of the hypertensive episode, almost coincident with the increase in HR, during which it lost its characteristic bursting pattern. Thereafter the RSNA resumed the usual cardiac rhythm at a level of activity slightly lower than at the end of the FRN.

The responses to the reopening were highly reproducible: the hypertensive and tachycardic episode was observed in all rats of group 3 (Table 2). The RSNA excitation at the peak of the BP increase was observed in four out of five rats. The mean changes in HR and BP that occurred after the reopening of the right kidney in the rats of group 3 are reported in Table 2. Shortly after the end of the experiment (i.e., 65 min after REOP) PRA was $57.5 \pm 7.7$ ng AI/ml per h ($n = 4$), which was highly statistically different from the PRA of groups 1 and 2 ($P < 0.001$).

### Effects of captopril: group 4 ($n = 7$)

The group 4 rats underwent bilateral nephrectomy in the same way as those in group 3, except that a nerve was not isolated and RSNA was not recorded. Hence in this group the time from anaesthesia to the beginning of the experimental protocol was shorter than in previous groups ($144 \pm 6$ min). In three group 4 rats 3 mg/kg captopril was injected intravenously half an hour before the reopening of the right kidney after 3 h of FRN. In all three animals REOP was only accompanied by a slight and slow increase in BP, mainly of the diastolic BP, and by a decrease in HR. The mean responses of these three group 4 rats and those of group 3 (no captopril) are contrasted in Table 2. Figure 5 is a representative example of the response in these three group 4 rats: at the closure of the right renal hilum (Fig. 5a) a slight but sudden increase in BP occurred,

**Fig. 4**




Analogue recording of the effects of reopening the functional right nephrectomy (FRN) for one animal from group 3. The bottom trace (renal sympathetic nerve activity, RSNA) shows the analogue output of the digital neural spike analyser preset to count the number of impulses/10 s.

Table 2 **Mean heart rate and blood pressure changes at the reopening of the right renal hilum for group 3 ($n = 5$) and group 4 ($n = 3$) rats**

| | Control | Reopening | | |
|---|---|---|---|---|
| | at 4h | peak | max change | time interval |
| Group 3 | | | | |
| HR | 364.1 ± 11.8 | 469.3 ± 20.6 | 105.1 ± 29.5 | 336.0 ± 73.1 |
| MBP | 109.8 ± 5.9 | 167.3 ± 11.3 | 57.1 ± 6.4 | 246.0 ± 91.7 |
| Group 4 | | | | |
| HR | 331.6 ± 15.3 | 323.9 ± 15.8 | −7.6 ± 4.9 | 590.0 ± 238.9 |
| MBP | 88.9 ± 8.4 | 103.3 ± 8.5 | 14.3 ± 3.4 | 590.0 ± 238.9 |

Control 4 h, mean control values immediately before reopening at time 4 h; Reopening peak, mean of the maximum response; Reopening change, mean of the maximum change compared with control values; Reopening time interval, mean time in seconds from the reopening to the peak change of the variable. HR, heart rate (bpm); MBP, mean blood pressure (mmHg). In group 4 3 mg/kg captopril was injected intravenously half an hour before reopening.

because of the exclusion of the renal vascular bed. Captopril injection (Fig. 5b) was followed by a slight decline in BP, mainly diastolic BP, an increase in pulse BP and a sharp, although modest, increase in HR. After the reopening procedure (Fig. 5c), only a slight and brief increase in BP was observed.

In the remaining four group 4 rats 3 mg/kg captopril was injected intravenously a few minutes after the reopening procedure, once the BP increase had occurred and achieved a steady state (average mean BP 1 min before REOP was 84.3 ± 2.4; average mean BP 5 min after REOP was 150.1 ± 4.5). The administration of the angiotensin-converting enzyme inhibitor was followed by an almost immediate fall in BP (average mean BP 2 min after captopril administration was 83.4 ± 18.5). Figure 6 shows a representative example of these experiments.

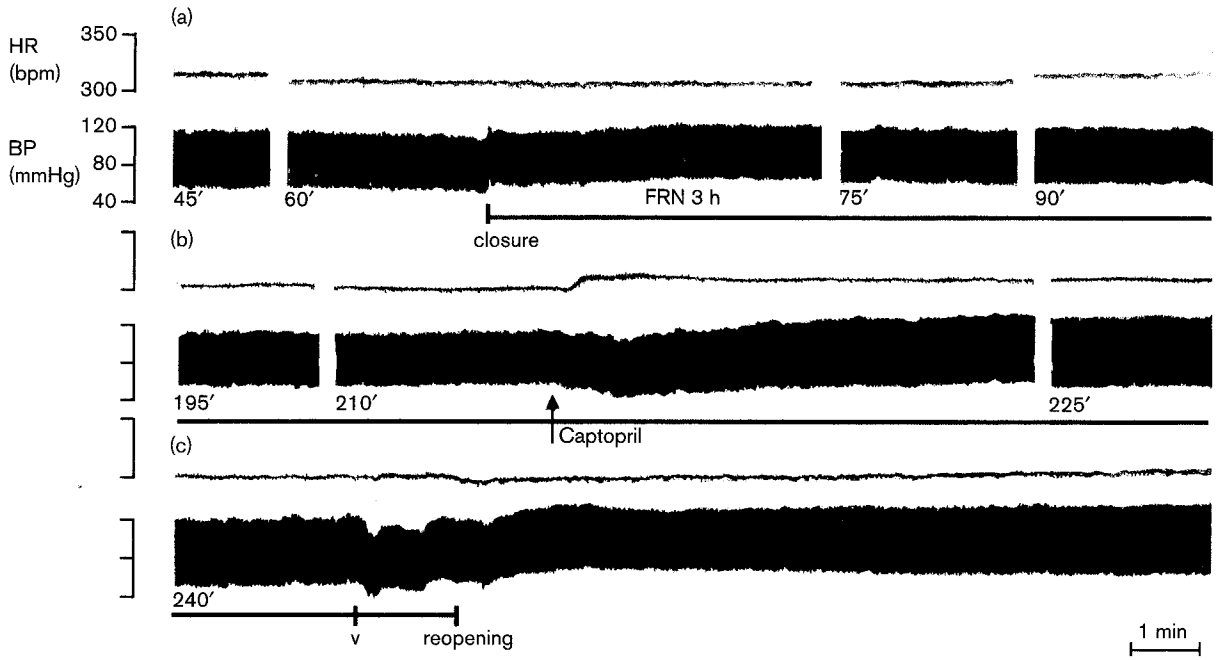
## Discussion

The following discussion is based on two main experimental observations: first, the RSNA spontaneously increased during 5 h of continuous recording in rats with the two kidneys intact and in rats with only one kidney remaining, whereas it progressively declined during the 3 h the remaining kidney was temporarily excluded by ligature of the hilum (FRN); and second, the reopening of the hilum of the previously excluded (and obviously ischaemic) remaining kidney was followed by a marked hypertensive and tachycardic response, by short lasting changes in RSNA and by a consistent increase in PRA.

### Spontaneous changes in RSNA and effects of mono- and bilateral nephrectomy

The increase in RSNA in group 1 and 2 rats and the decrease in RSNA during FRN in group 3 occurred

**Fig. 5**




Analogue recording of heart rate (HR) and arterial blood pressure (BP) for one animal from group 4. The numbers at the beginning of each strip below the BP tracing indicate the time period of the recording with respect to the beginning of the experimental protocol. During the first 45 min, the 102 min between the end of (a) and beginning of (b), and the 13 min between (b) and (c), no changes in HR and BP were observed. (a) at 63 min the start of functional right nephrectomy (FRN) is marked by a tick bar below the BP tracing and by 'closure'. (b) 213 min from the beginning of the experiment, i.e. 2 h and 30 min from the start of FRN, 3 mg/kg captopril was injected intravenously. (c) 243 min from the beginning of the experiment, i.e. 3 h from the start of FRN, first the renal vein (first vertical mark on FRN bar: v) then the renal artery ('reopening') were untied.

without any concomitant significant change in T, RB, HR and mean BP. Moreover, the haematocrit data indicate that blood volume was unlikely to have changed during any of the experiments and that in rats of group 3 it was probably less expanded than in rats with one or both kidneys intact. In fact precautions were taken not to expand blood volume in all three groups, and during functional nephrectomy the intravenous infusion of saline was halved. In the light of the constancy of T, RB, HR, BP and haematocrit it is unlikely that changes in RSNA can be ascribed to reflex influences from arterial baro- and chemoreceptors or from cardiopulmonary receptors [2,13–16].

The increase in RSNA was caused by an increase in the number of bursts per cardiac cycle and an increase in the amplitude of the bursts, whereas during FRN opposite changes occurred in the number of bursts/sec. As it is known that the amplitude (and the duration) of the trains of impulses is directly related to the number of active neural elements [4–6], it is reasonable to assume that the increase in RSNA reflects not only the activation of already spontaneously active neurons, but also the recruitment of previously silent neural units, whereas during bilateral nephrectomy neurons that had been spontaneously active became silent.

Thus, the main question we set out to investigate has been answered, and the conclusion can be drawn that in the anaesthetized experimental animal the normal kidney exerts a supportive function on the activity of the sympathetic nervous system, or at least of the sympathetic fibres directed to the kidneys.

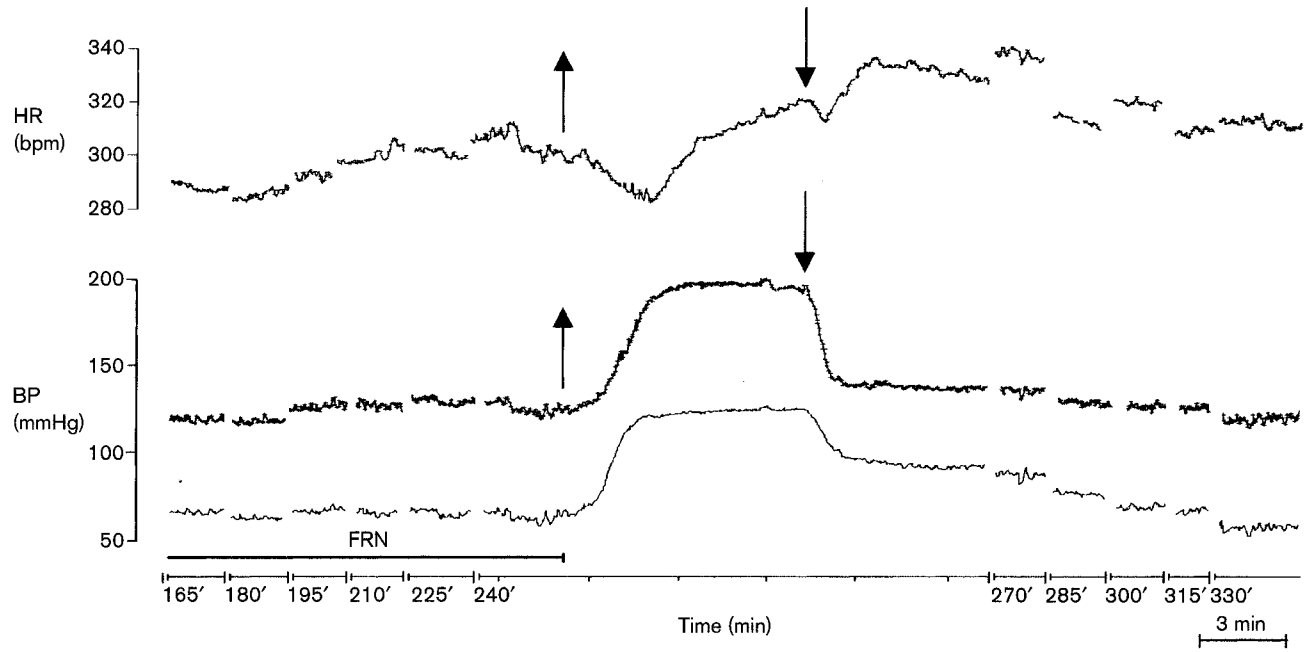
### Mechanisms of the kidney effects on sympathetic nerve activity

The question of the mechanism by which the kidney can exert this sympathetic activating role is more difficult to answer. As anticipated in the introduction, nephrectomy does not allow a definite distinction to be made between the respective roles of renal sensory innervation and of substances released from the kidneys. Despite this limitation we shall briefly outline the conclusions that might be drawn and the hypotheses that can be raised by analysing our data in the context of available experimental evidence.

#### *Renal receptors*

In pathophysiological conditions, such as chronic renal failure in rats [17–19], intrarenal infusions of ischaemic metabolites [20] and of bradykinin [21,22], renal artery stenosis in the rat [23,24], experimental models of hypertension [25–27] and in chronic renal failure pa-

**Fig. 6**




Effects of captopril on the haemodynamic response to the reopening procedure. Time base: the numbers below the abscissa indicate the starting time of the recording periods with respect to time 0. Heart rate (HR) and arterial blood pressure (BP) tracings are shown for a period of 2 h and 45 min. These variables were recorded for 2 min at 15-min intervals from time 165' (2 h 45 min) to time 330' (5 h 30 min), except at time 240' (4 h), when a continuous recording of 17 min is shown. Functional right nephrectomy (FRN) was maintained for 3 h. Upward arrow: reopening of the right renal hilum after 3 h of FRN. Downward arrow: 3 mg/kg captopril was injected intravenously. BP and HR tracings have been drawn by the computer program.

tients [11] the available evidence favours an excitatory influence of renal receptors, mainly chemoreceptors [8–10,28,29], on central and peripheral sympathetic activity. These data have usually, but not always, been confirmed by the reflex effects elicited by electrical stimulation of renal afferent nerves [2]. However, in normal physiological conditions both excitatory [30–33] and inhibitory reflex influences have been reported [1,2,34,35], and in experimental conditions similar to those of the present investigation the afferent nerve activity of R2 chemoreceptors was shown not to increase spontaneously during a period of 5 h (Recordati, unpublished observation).

Apart from these considerations, the hypothesis that the increase in RSNA in group 1 and 2 rats was mostly the result of a viscerovisceral excitatory reflex initiated by the activation of renal chemoreceptors is hardly tenable as in group 2 rats the nerves from the only remaining kidney were almost entirely interrupted to prepare an efferent nerve bundle for recording. The possibility that the small and statistically not significant difference in RSNA between group 1 (left renal nerve intact) and group 2 represents a small reflex-initiated component cannot be excluded on the basis of the present experiments.

### *Role of renal substances*

Among the substances that a kidney may release into the systemic circulation, angiotensin II, the main active element of the renal renin–angiotensin system, is able to activate or facilitate the sympathetic nervous system at various levels [6,7,36–46]. As it is known, moreover, that pentobarbital anaesthesia and surgical stress may stimulate renin release [37,39] it is possible that the spontaneous increase in RSNA observed in rats with one or both kidneys intact might be caused by an increase in renin release and in plasma angiotensin II concentration. This hypothesis is consistent with our observation that during bilateral nephrectomy, which abolishes renin release into the systemic circulation, RSNA spontaneously declined.

Anaesthesia *per se* has also been shown to be responsible for a widespread increase in sympathetic nerve activity [47–49]. The same anaesthetic agent was used throughout our experiments, hence it cannot explain the different responses of groups 1 and 2 compared with those of group 3 rats. Furthermore, we have used pentobarbital anaesthesia, a single bolus injection of which has recently been shown to produce a decline in RSNA rather than an excitatory response [50].

These observations lead us to favour the hypothesis that the increase in RSNA in rats with one or both kidneys intact was the result of a central and/or peripheral sympathetic system stimulation exerted by angiotensin II generated by the increased renin release induced by pentobarbital anaesthesia and surgical stress; in parallel, the decrease observed during bilateral nephrectomy is likely to be caused by the complete suppression of the renin release. In addition, although our data do not allow us to exclude the influence of other substances released by the kidneys, the hypothesis of involvement of the renin–angiotensin system is supported by the moderately high PRA levels measured at the end of the recording period in groups 1 and 2 and is further substantiated by the effects of the reopening of the only remaining and completely ischaemic kidney.

### Effects of the reopening of the excluded (ischaemic) kidney

The responses to the reopening procedure were highly reproducible: BP and HR in all five rats and RSNA in all but one of the animals underwent similar changes.

Possible explanations for the observed brief excitation of RSNA at the peak of the BP rise include, first, stimulation by the BP increase of visceral receptors located on the adventitia of the thoracic aorta with afferent fibres in the sympathetic nerves; these are known to participate in sympathosympathetic spinal reflexes [51–53]. An additional and more likely hypothesis is that an excitatory sympathetic effect at central or ganglionic level results from the overflow into the systemic circulation of a substance released by the ischaemic kidney at the time of its reperfusion. The late decline in RSNA might be the result of intense baroreceptor activation from the marked increase in BP.

The increases in BP and HR after the reopening of the hilum of the right kidney were very large and consistent, and persisted well above the control level for at least 1 h after the reopening procedure; they were accompanied by very high levels of PRA. As a bolus intravenous injection of captopril either prevented or reversed these hypertensive and tachycardic effects, it is evident that they were caused by a sudden release of renin and by the consequent generation of angiotensin II.

In fact only a very large amount of angiotensin II would be able to produce hypertension with simultaneous tachycardia. A similar effect has been described for exogenous angiotensin II injected directly into the vertebral artery of a dog [54] and the tachycardia accompanying this hypertension has been attributed to the withdrawal of cardiac parasympathetic tone in response to a central action of the exogenous angiotensin II on cardiovascular brainstem centres [55]. Data on the acute effects of the release of endogenous angiotensin II on RSNA, BP and cardiac function were not, to our knowledge, available in advance of our experimental observations.

## Conclusions

We have shown that the kidneys exert a supportive and facilitatory function on the renal efferent sympathetic activity in the normal anaesthetized rat.

Our observations can tentatively be explained by the interactions between the renal renin–angiotensin and sympathetic nervous systems [3,7]. In rats with one or both kidneys intact the anaesthesia and surgical stress would stimulate renin release from the kidneys and angiotensin II would in turn act centrally and peripherally to sustain sympathetic tone and increase RSNA during the 5 h of control recording. During bilateral nephrectomy, because of the lack of endogenous angiotensin II, RSNA progressively declines. Upon the reopening of the only remaining kidney a consistent amount of angiotensin II is released, which acts both centrally and peripherally to produce hypertension and tachycardia and a transient increase in RSNA, probably shortened by baroreceptor activation. For renal sensory innervation, under normal physiological conditions a tonic supportive role on RSNA needs to be further substantiated by unequivocal experimental evidence; however, the hypothesis reported above does not exclude the possibility that when intrarenal conditions exist to sustain an acute or chronic excitation of renal sensory elements, this excitatory afferent input may contribute to modify, through a reflex action, sympathetic activity and blood pressure [17–27,30]. It has been clearly demonstrated that renal chemoreceptors are very resistant to prolonged anoxia [8–10].

Finally, a consideration about the effects of the reopening of the ischaemic kidney. Since the first demonstration by Tigerstedt and Bergman [56] of the hypertensive effects of the injection of extracts of renal cortex in 1898, an impressive amount of work has been done on the renin–angiotensin system and many models of renal experimental hypertension have been designed. All of them, however, require some time before the hypertension becomes manifest, and thus should be considered chronic models of experimental renal hypertension. The effects of the reopening of a kidney made completly ischaemic for a few hours may thus represent the first evidence of an experimental acute hypertension with tachycardia caused by the acute release of renin and the consequent generation of angiotensin II.

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