DCNP sensitivity curves, which showed a pattern for each drug intermediate between that of phenol and dopamine (Fig. 1), show the enzyme to be similar to that of the platelet. Thus, all the evidence obtained so far supports the classification of placental PST into P and M forms, and suggests that they have similar properties to those elsewhere in the body. Whether maternal platelet activity reflects that in the placenta cannot be taken for granted, however, for a substantial proportion of a placental enzyme is presumably foetal in origin.

How may the findings reported here be of clinical relevance? Salbutamol is used to suppress premature labour and other β_2-adrenergic stimulating drugs used in this way, such as fenoterol and ritodrine, may also be substrates for PST. Paracetamol and salicylamide are also widely used in self-medication so that the activity of the placental enzyme will, to some extent, determine the extent to which they traverse the placental barrier. Thus, the two forms of the enzyme may help to regulate the amount of free drug gaining access to the fetus. This factor is of particular importance when questions of teratogenicity are raised, not only in its florid, anatomical forms, but also on a more subtle, behavioural level.

Bernhard Baron Memorial Research Laboratories and Institute of Obstetrics and Gynaecology
Queen Charlotte's Maternity Hospital, Goldhawk Road London W6 0XG, U.K.

R. J. SODHA*
VIVETTE GLOVER
M. SANDLER†

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* Present address: Universitätsspital Zürich, Department für Frauenheilkunde, C-Nord, Frauenklinikstrasse 10, 8091 Zürich, Switzerland.
† Author to whom correspondence should be addressed.

Biochemical Pharmacology, Vol. 32, No. 10, pp. 1657–1660, 1983.
Printed in Great Britain.

0006-2952/83 $03.00 + .00

The influence of a unilateral nephrectomy on the kallikrein activity of the remaining kidney and on the urinary kallikrein excretion in rats

(*Received* 10 *September* 1982; *accepted* 13 *December* 1982)

It has been suggested that the renal kallikrein-kinin system is responsible for the reduction of the tubular sodium reabsorption in a kidney after excision of the contralateral. This suggestion was based on the finding of increased kallikrein excretion after subtotal nephrectomy in rats [1]. It has been reported, however, that unilateral nephrectomized rats excrete less kallikrein than controls [2, 3]. To clarify this discrepancy, we investigated the influence of a unilateral nephrectomy on the urinary kallikrein excretion as well as on the kallikrein activity of the remaining kidney.

Methods. Male Wistar rats (200 g body weight) were anesthetized with ether. The left kidney was excised through a flank incision. Control rats were sham operated. Ten animals were alloted to each of five experimental (UNx) and five control (C) groups (100 animals). The rats were placed in collective cages and kept in a room at constant temperature and humidity (22°, 50%) with a 12 hr light–dark cycle. After a 3 days accommodation period to stainless steel metabolic cages, 24 hr urine was collected (in plastic beakers at room temperature) from a C and a UNx group 8, 15, 30, 60 and 90 days after surgery. At the end of the urine collection, the animals were anesthetized with pentobarbital (40–50 mg/kg body weight, i.p.) and a carotid artery was cannulated to measure blood pressure by means of a strain gauge. Then, the kidney(s) were excised. Kidneys and urine were kept frozen until assayed. Protein concentration in kidney homogenates was measured by the method of Lowry *et al.* [4], and creatinin in urine by the method of Popper *et al.* [5]. Urinary free aldosterone was estimated with a radioimmunoassay [6]. Plasma and urinary osmolarity was measured with a freezing point osmometer, and Na^+ and K^+ with an internal standard flame photometer. To determine the kallikrein activity, appropriately diluted urine and whole kidney homogenates were incubated with D-val–leu–arg–paranitroanilide (S2266, KabiDiagnostica, Stockholm, Sweden) for 120 min at 37° [7, 8]. A kallikrein unit (U) is the amount of enzyme capable of hydrolysing 1 μmole of substrate per

Table 1. Urinary parameters from sham operated and unilaterally nephrectomized rats

Days after surgery	$\dot{V}$ (ml/day)		$[Na^+]_u \cdot \dot{V}$ (mmoles/day)		$[K^+]_u \cdot \dot{V}$ (mmoles/day)		Na^+/K^+	
	C	UNx	C	UNx	C	UNx	C	UNx
8	34 ± 2	31 ± 2	5.9 ± 0.3	5.9 ± 0.5	7.5 ± 0.4	7.2 ± 0.5	0.80 ± 0.02	0.81 ± 0.01
15	23 ± 2	32 ± 3*	5.0 ± 0.3	5.1 ± 0.5	6.6 ± 0.5	6.5 ± 0.5	0.77 ± 0.02	0.77 ± 0.02
30	21 ± 1	27 ± 1***	3.9 ± 0.2	5.0 ± 0.4*	5.0 ± 0.2	5.3 ± 0.5	0.79 ± 0.01	1.01 ± 0.12
60	24 ± 3	22 ± 2	3.1 ± 0.2	3.2 ± 0.2	4.3 ± 0.3	4.4 ± 0.2	0.73 ± 0.02	0.74 ± 0.03
90	29 ± 2	28 ± 2	3.8 ± 0.2	3.8 ± 0.2	4.8 ± 0.2	4.8 ± 0.2	0.80 ± 0.02	0.80 ± 0.01

Values are $\bar{x}$ ± S.E. $\dot{V}$ = 24 hr urine volume. $[Na^+]_u \cdot \dot{V}$ = sodium excretion. $[K^+]_u \cdot \dot{V}$ = potassium excretion. Na^+/K^+ = sodium to potassium ratio in urine. Significances: * $P < 0.05$, *** $P < 0.001$.
C = sham operated rats. UNx = unilaterally nephrectomized rats.

min. Daily excretions were calculated by multiplying concentrations or activities by the 24 hr urine vol. Results are given as means ± S.E. The difference between groups was assessed with the Student's *t*-test (two tails) and correlations between parameters by linear regression analysis.

Results. UNx rats weighed less than C animals only 8 (UNx: 214 ± 2 g, C: 225 ± 4, $P < 0.05$) and 90 days after surgery (UNx: 416 ± 10 g, C: 449 ± 12 g, $P < 0.05$). The kidney which remained *in situ* after the unilateral nephrectomy was heavier than the homolateral of C rats from day 8 (UNx: 1.15 ± 0.03 g, C: 0.89 ± 0.03 g, $P < 0.001$) to day 90 (UNx: 1.78 ± 0.03 g, C: 1.21 ± 0.04 g, $P < 0.001$). The total kidney weight, however, remained reduced up to 90 days after surgery (UNx right: 1.78 ± 0.03 g, C right + left: 2.42 ± 0.07 g, $P < 0.001$). The protein concentration of kidney homogenates from UNx rats did not differ from C. Fifteen days after UNx, the kidney remaining *in situ* had less kallikrein activity (RKal, mU/mg protein) than the homolateral kidney of controls (Fig. 1, lower panel).

The total RKal (mU/g kidney weight × kidney weight) of the UNx rats was about 50% that of C animals (both kidneys added) up to day 60 after surgery (UNx: 103 ± 8 mU; C: 219 ± 9 mU, $P < 0.001$). It was still reduced by day 90 (UNx: 219 ± 21 mU, C: 362 ± 23 mU, $P < 0.001$).

Fifteen and 30 days after unilateral kidney excision, UNx rats had a higher urine vol. than their controls (Table 1). Sodium excretion decreased progresively up to 60 days after operation. Since the fall was delayed in the UNx rats, they excreted more Na^+ than C rats by day 30 after surgery (Table 1). The K^+ excretion also decreased with age (Table 1). Neither the Na^+/K^+ ratio in urine (Table 1), nor the creatinin excretion of UNx rats differed from C (Table 2).

UNx rats excreted less kallikrein (UKalV) than C up to 30 days after surgery (Fig. 1, upper panel). However, when calculated per g of kidney, only the rats studied 15 and 30 days after unilateral nephrectomy had reduced UKalV (Table 2). The UKalV of C rats increased with age (8 days 0.90 ± 0.11 mU/day, 90 days: 1.25 ± 0.11 mU/day $P < 0.05$), reaching a maximum by day 60 after unilateral nephrectomy. By then the rats had a body weight of 360 ± 4 g. UKalV remained constant when calculated per g of kidney (Table 2) or per kg of body weight (8 days: 3.30 ± 0.16 U/kg BW·day, 90 days: 2.84 ± 0.30 U/kg BW·day).

None of the UNx groups had a systolic blood pressure different from C (not shown). The negative free water clearance ($-C_{H_2O}$) was lower than C 15 days after UNx (Table 2). The excretion of free aldosterone, which was only measured in the rats studied 15 and 30 days after operation, did not change (Table 2). An inverse relationship between sodium and kallikrein excretion was observed (UNx 30 days vs C 30 days; UNx and C 60 days vs all other, Tables 1 and 2). The same was true when data from all animals was taken into account (*r*: −0.37, N: 96, $P < 0.001$).The UKalV was positively related to $-C_{H_2O}$ (UNx 15 days vs C 15 days, Tables 1 and 2; all animals, *r*: 0.29, N: 91, $P < 0.01$) and inversely related to the urine vol. (UNx vs C 15 and 30 days, Tables 1 and 2; all rats, *r*: −0.35, N: 92, $P < 0.001$), as well as to the Na^+/K^+ ratio (UNx vs C 30 days; all rats, *r*: −0.24, N: 96, $P < 0.05$). The UKalV was neither correlated to the potassium excretion (*r*: −0.11, N: 96, $P < 0.1$) nor to the osmolar clearance (*r*: 0.12, N: 91, $P > 0.1$).

A correlation between the RKal of the right and that of the left kidney was found in C rats. The UKalV of all rats taken together correlated better with the total RKal (*r*: 0.40, *n*: 95, $P < 0.001$), than with the activity per g of kidney (means of both kidneys in controls, *r*: 0.21, *n*: 95, $P < 0.005$).

Discussion. The finding that UKalV in rats decreases after unilateral nephrectomy contradicts the data of Oza *et al.* [1] and confirms those of Vio *et al.* [2] and Albertini *et al.* [3]. It should be pointed out, however, that in our laboratory UKalV was still reduced (rather than normal) [2, 3] 30 days after unilateral nephrectomy. Since the kal-

Table 2. Data from sham operated (C) and unilaterally nephrectomized (UNx) rats

Days after surgery	$U_{creat} \cdot \dot{V}$ (mg/day)		$U_{aldo} \cdot \dot{V}$ (ng/day)		C_{H_2O} (ml/day)		UKal (mU/day·g kidney)	
	C	UNx	C	UNx	C	UNx		
8	28 ± 2	25 ± 2	—	—	−48 ± 6	−40 ± 6	509 ± 48	522 ± 74
15	20 ± 1	21 ± 2	4.76 ± 0.52	6.47 ± 1.01	−62 ± 4	−47 ± 5*	549 ± 49	316 ± 24***
30	19 ± 1	19 ± 1	7.63 ± 2.15	5.44 ± 1.08	−65 ± 3	−65 ± 3	533 ± 72	334 ± 35*
60	22 ± 2	21 ± 1	—	—	−79 ± 13	−52 ± 6	695 ± 107	840 ± 130
90	23 ± 3	25 ± 2	—	—	−69 ± 4	−64 ± 8	524 ± 51	605 ± 89

Values are $\bar{x}$ ± S.E. $U_{creat} \cdot \dot{V}$ = 24 hr creatinine excretion. $U_{aldo} \cdot \dot{V}$ = aldosterone excretion.
C_{H_2O} = free water clearance. UKal = kallikrein activity per ml of urine × 24 hr urine vol.
Significances: * $P < 0.05$, *** $P < 0.001$.

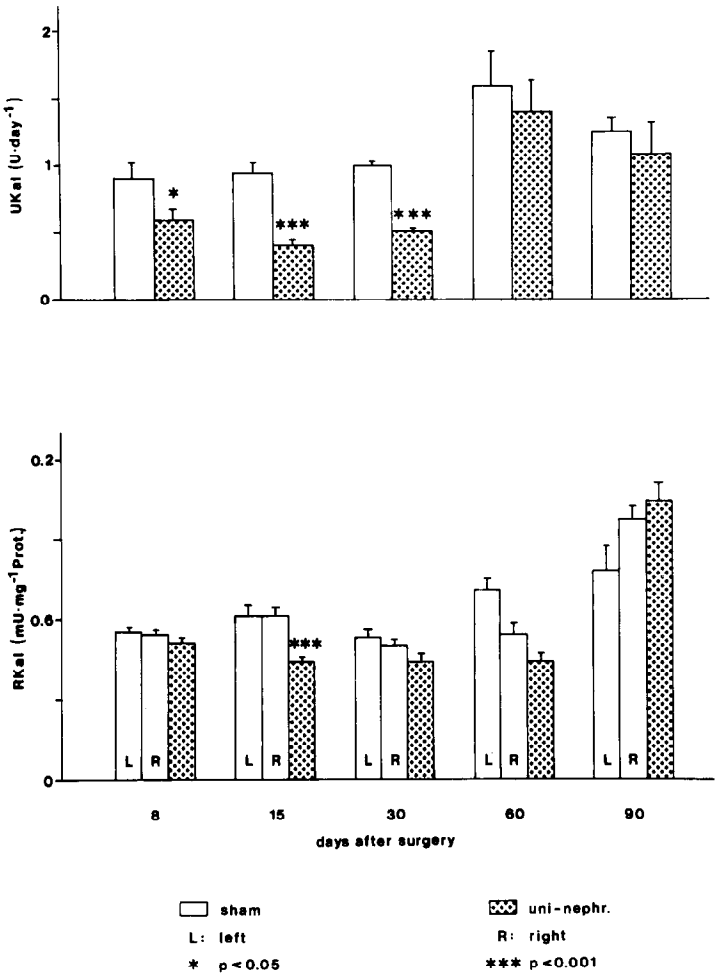

Fig. 1. Urinary kallikrein excretion (UKal) and renal kallikrein activity (RKal) of sham operated and unilaterally nephrectomized (uni-nephr.) rats measured at various time intervals (abscissa) after surgery.

likrein excreted per g of kidney by UNx rats 8 days after surgery did not differ from that of C, it is likely that the lower UKalV found at this time simply depends on the elimination of kallikrein producing cells.

The finding of a diminished excretion of kallikrein per g of kidney 15 and 30 days after UNx suggest an actual reduction of the kallikrein produced by the remaining kidney of these animals. That the kidney of the 15 days UNx rats produces less amount of this enzyme is also indicated by its lower RKal. It has been suggested that renal kallikrein is under the control of mineralocorticoids [9, 10]. Perhaps the low UKalV in the 30 days UNx group coexisted with a subnormal plasma aldosterone concentration. Their higher (though not significant) Na^+/K^+ ratio in urine might suggest this. However, the excretion of free aldosterone was not reduced. An involvement of this hormone cannot be totally ruled out, because we did not measure the aldosterone metabolytes tetrahydroaldosterone and aldosterone glucoronide. The normal aldosterone and K^+ excretion, as well as unaffected Na^+/K^+ ration in the urine of 15 days UNx rats, suggest that a factor other than reduced aldosterone was responsible for their lower UKalV. Their enhanced urine vol., along with the lower $-C_{H_2O}$, suggest that these rats had a reduced plasma concentration of antidiuretic hormone (ADH). Since this hormone can stimulate the urinary kallikrein activity [11], its suppression may explain a reduced UKalV. This possibility is supported by the correlation found between UKalV and $-C_{H_2O}$. However, neither the reduction of ADH in these animals, nor the stimulatory effect of this hormone on the kallikrein excretion are certain [12, 13]. Suppression of kidney kallikrein in unilateral nephrectomized rats might be involved in the enhancement of the fractional excretion of sodium and water by the sole kidney.

Several conclusions may be drawn from the results reported here. (1) Kallikrein excretion increases with age; thus, results of experiments performed with rats should be expressed per g of kidney weight or kg of body weight. (2) The significant correlation found between right and left RKal in normal rats supports the concept that extrarenal factor(s) regulate the activity of the enzyme. (3) The decrement of UKalV brought about by a reduction of the renal mass depends partly on elimination of kallikrein producing cells and partly on inhibiting mechanism(s). (4) The relationship found between UKalV and Na^+ excretion, diuresis and $-C_{H_2O}$ suggest that the physiological role of renal kallikrein is to conserve water and sodium.

Summarizing, the influence of a unilateral nephrectomy on the urinary kallikrein excretion (UKalV) and on the kallikrein activity (RKal) of the remaining kidney was investigated 8, 15, 30, 60 and 90 days after surgery. UKalV of sham operated rats (C) increased with age, but remained constant when it was related to kidney or body weight. UKalV correlated better with the total RKal than with its activity per g of kidney or mg of protein. Unilateral nephrectomized rats (UNx) excreted less kallikrein than C rats

up to 30 days after surgery. The kallikrein excretion of 15 and 30 days UNx rats was lower than C even when expressed per g of kidney weight. Only 15 days UNx rats had diminished RKal. UNx rats had normal blood pressure (all groups), increased urine vol. (15 and 30 days), increased natriuresis (30 days) and decreased negative free water clearance (15 days). UKalV from all animals was inversely related to sodium excretion, Na^+/K^+ ratio, as well as to urine vol., and directly related to the negative free water clearance.

Acknowledgements—This work was supported by the Deutsche Forschungsgemeinschaft (SFB 90). The technical assistance of Mrs. G. Breypohl, Miss J. Kopatsch and Miss. H. Seeger is gratefully acknowledged.

Department of Pharmacology
University of Heidelberg
Im Neuenheimer Feld 366
D-6900 Heidelberg, F.R.G.

MARCOS MARIN-GREZ*
GASTON SCHAECHTELIN

* To whom reprint requests should be addressed.

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Biochemical Pharmacology, Vol. 32, No. 10, pp. 1660–1663, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + 0.00

Uptake of [^{3}H]nitrendipine into cardiac and smooth muscles

(*Received* 18 *August* 1982; *accepted* 23 *December* 1982)

Nitrendipine, an asymmetrically substituted derivative of 1,4-dihydropyridine, is a new cardiovascular agent belonging to the class of calcium antagonists [1]. This drug causes coronary vasodilation and exerts an antihypertensive action [2].

The present study was undertaken to probe further the mechanism of action of nitrendipine, in particular its ability to cross the surface membrane of the muscle cells. The uptake of nitrendipine by cat ileal smooth muscle and by chick embryonic ventricular muscle was examined.

Cat ileal smooth muscle strips were prepared according to the method of Sperelakis [3]. A long segment of ileal small intestine was removed from anesthetized cat and immediately immersed in ice-cold Ringer solution. The lumen of the cat intestine was washed thoroughly with ice-cold Ringer solution using a syringe. A segment (3–4 cm) of intestine was mounted on a 5-ml pipet for dissection. The outer serosa and the longitudinal muscle layer were carefully removed with a pair of fine forceps. Then, the intestinal tube was inverted and remounted on the pipet. The layer of mucosa and submucosa was carefully separated from the circular muscle layer and stripped off. The remaining muscle tube contained relatively pure circular smooth muscle and was dissected into rings 5 mm wide, which were stored at 0° in Ringer solution before use.

Embryonic hearts were removed from fertilized chicken eggs (White Leghorn, Babcock strain) at 9 days of age. The ventricles were then dissected from the hearts, washed and stored in ice-cold Ringer solution before use.

The composition of the Ringer solution for [^{3}H]nitrendipine uptake was: 145 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 20 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes) (pH 7.4) and 10 mM glucose. Nitrendipine (Miles Laboratories) was dissolved in absolute ethanol and added to give final concentrations of 10^{-9}–10^{-6} M. Appropriate amounts of [^{3}H]nitrendipine (88 Ci/mmole, ICN Pharmaceuticals) were added.

The muscles were incubated at 37° and, at appropriate time intervals, the muscle samples were removed from the incubation medium and blotted dry. To wash drugs taken up in the interstitial fluid space of the muscle preparations, the muscles were incubated for 10 min in ice-cold Ringer solution [4]. The muscle samples were again blotted, weighed (for the smooth muscle only), and dissolved in 0.1 N NaOH at 60° overnight. The radioactivity of the solubilized muscle sample was determined by liquid scintillation counting, and the protein content was determined by the method of Lowry *et al.* [5].

For efflux experiments, ileal muscle was loaded with 10^{-6} M nitrendipine or verapamil (1 μCi/ml) for 3 hr [4]. The muscles were then removed, blotted, dried, and passed through a series of efflux tubes containing 1 ml of Ringer's solution. The muscle was moved sequentially from tube to tube at 0.25, 0.5, 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, and