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Renin release from the ischaemic kidney

JOAN S. LONG AND W. B. SEVERS

Department of Pharmacology, College of Medicine, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033

Summary

1. We have confirmed that spinal section, and also pithing, inhibits the pressor response associated with the release of renin that follows re-establishment of circulation to the ischaemic rat kidney. Brain transections at bulbar and midthalamic levels did not modify the blood pressure elevation.

2. Several pharmacological antagonists of the sympathetic nervous system did not modify the blood pressure response. These observations are not consistent with the view that a neural element is necessary for renin release.

3. Constant flow perfusion of the ischaemic kidney in the intact and spinal sectioned rat was performed to evaluate haemodynamic factors involved in renin release. The pressor response was present in spinal sectioned animals under these conditions.

4. These results suggest that the nervous system is necessary to maintain adequate blood flow for renin 'washout' into the systemic circulation rather than to release renin from juxtaglomerular cells.

Introduction

Taquini & Braun-Menendez (1941) first demonstrated in dogs that the blood pressure elevation following re-establishment of blood flow to an ischaemic kidney resulted from the passage of renin into the systemic circulation. Further studies by Taquini, Blaquier & Taquini (1964) revealed that plasma renin concentration was very high several minutes after recirculation but rapidly diminished thereafter. According to these and other related studies, Taquini *et al.* (1964) concluded that a period of prolonged ischaemia results in an outflow of renin from the cells and that this outflow appears in the renal vein after the circulation is re-established. However, under similar conditions of renal ischaemia in the rat Hayden & Targett (1971a, b) suggest that the nervous system is necessary for renin release. These authors reported that cervical spinal section inhibits the rise in plasma renin activity and the concurrent elevation in blood pressure observed after re-establishing circulation to the ischaemic kidney. The purpose of the present paper was to characterize specifically the neural requirements for renin release from the ischaemic kidney.

Methods

Procedure for producing an ischaemic kidney

The procedure of Hayden & Targett (1971a) was used. Male albino Sprague–Dawley rats weighing between 300 and 450 g were anaesthetized with ether and

the left kidney was exposed via a retroperitoneal incision. The renal pedicle was isolated and carefully cleared of fat and connective tissue. The renal artery was gently separated from the renal vein and ureter and a 2 mm wide artery clamp was applied to the artery. The incision was then closed with wound clamps and the animals allowed to recover in individual cages. Approximately 3·5 h later the rats were anaesthetized with sodium pentobarbitone, 40 mg/kg i.p., and the femoral artery was cannulated for recording blood pressure. Approximately 4 h after the clamp was positioned the incision was opened and the clamp removed. Blood pressure was continuously recorded on a polygraph. About 1 h after the clamp was removed recirculation through the ischaemic kidney was evaluated by the injection of 10 μg adrenaline in 0·1 ml or, on occasion, of 20 μg phenylephrine in 0·1 ml, directly into the kidney pulp. Pressor responses and tachycardia produced by similar injections were used by Hayden & Targett (1971a) as a test for re-establishment of renal circulation. This basic ischaemic kidney preparation was studied in additional groups of rats receiving various surgical or drug treatments to be described.

Neural sections

In neural transection experiments rats were positioned in a stereotaxic head holder with the upper incisor line 5–8 mm above the intra-aural line. The following transections were made:

1. *Cervical spinal section.* The cervical spinal cord was exposed by a dorsal incision into the neck. The dura was cut and the spinal cord transected between C_1 and C_2.

2. *Bulbar section.* The cisterna was exposed by a dorsal incision into the neck. A majority of the supraoccipital bone was removed and the dura cut. The cerebellum was elevated slightly and the blade was obliquely passed in an anterior direction.

3. *Midthalamic section.* The skull was exposed and bone was removed laterally (2–4 mm posterior to the bregma). The underlying dura was opened and the transection was made laterally in a plane 3 mm posterior to the bregma.

4. *Spinal pith.* The cisterna was isolated by a dorsal neck incision, the dura cut, and a 2 mm diameter pointed rod was inserted into the vertebral column at this level and the spinal cord pithed. The brain remained intact. The rod was left in the vertebral column for the remainder of the experiment to minimize haemorrhage. Approximately 5 ml of whole heparinized rat blood was infused intravenously as necessary to maintain a stable blood pressure.

The animals received intermittent positive pressure respiration when necessary. All transections were performed about 30–45 min before releasing the renal arterial clamp. At the end of brain sectioning experiments the brain was removed and fixed in 10% formalin and the path of the section was located by gross dissection.

Pharmacological studies

Various drugs whose dose regimens are listed in Table 1 were studied in the ischaemic kidney preparation. In experiments with autonomic agents, appropriate agonists (isoprenaline, phenylephrine, histamine, 5-hydroxytryptamine, or tyramine), were administered intravenously approximately 1 h after clamp release.

TABLE 1. *Dosage regimens of pharmacological agents*

Drug	Base dose (mg/kg)	Route of administration	Pretreatment time‡ (prior to clamp release)
Phentolamine	5·0	i.v.	15 min
Phenoxybenzamine*	5·0	i.p.	24 h
	2·5	i.p.	6 h
Propranolol	7·5	i.v.	15 min
LB-46	1·0	i.r.†	when clamp applied
	1·0	i.v.	15 min
Atropine+hexamethonium	5·0	i.v.	15 min
	10·0		
Diphenhydramine	25·0	i.p.	15 min before renal clamp 15 min
Methysergide*	0·5	i.p.	10 min
Reserpine	5·0	i.p.	24 h
	2·5	i.p.	8 h
6-Hydroxydopamine*	50·0	i.p.	48 h
	25·0	i.p.	24 h
Guanethidine (as sulphate)	15·0	i.p.	24 h, 6 h

* Vehicles used for these compounds were: phenoxybenzamine, 48·5% v/v absolute alcohol, 0·5% v/v hydrochloric acid in redistilled propylene glycol; methysergide, 0·601 M tartaric acid in 0·9% w/v NaCl solution; 6-hydroxydopamine, 0·1% ascorbic acid in 0·9% w/v NaCl solution. † i.r.=injection into the ischaemic kidney pulp. ‡ If more than one time indicated, same dose was repeated unless otherwise specified.

The efficacy of 6-hydroxydopamine treatment was evaluated by renal noradrenaline assays (Shellenberger & Gordon, 1971) in separate rats. These data were used to evaluate respectively the β-adrenoceptor blocking, α-adrenoceptor blocking, antihistamine, anti-5-hydroxytryptamine, and noradrenaline depleting activities of the drugs used. All intravenous (i.v.) and intraperitoneal (i.p.) injections were administered in 0·9% w/v NaCl solution (saline) in a dose volume of 1 ml/kg unless otherwise stated.

Kidney phenol treatment

At the time of clamping the renal artery the adventitia was carefully stripped and the renal pedicle, artery and vein were painted with 1% phenol solution. In another group of similarly prepared rats the effect of papaverine hydrochloride was evaluated. One milligramme of the drug was injected into the kidney pulp just after clamping the renal artery and 15 mg/kg was administered i.v. 5 min prior to clamp release.

Ureter ligation

At the time of clamping the renal artery, the ureter from the ischaemic kidney was isolated and ligated.

Cross perfusion experiments

A donor rat was prepared with an ischaemic kidney as described. Before removal of the renal arterial clamp, the renal vein draining the ischaemic kidney was connected to a jugular vein of a recipient rat also anaesthetized with pentobarbitone (40 mg/kg, i.p.). The bridge for the connexion was about 6 inches of PE-90 tubing initially filled with heparinized saline, 1,000 units/ml. Blood pressure from both rats was recorded from femoral arterial catheters. An intravenous infusion of heparinized whole rat blood (0·3 ml/min) was started in the donor rat

when the renal arterial clamp was removed. This volume approximated the quantity of blood shunted from the donor renal vein to the jugular vein of the recipient animal.

Constant flow perfusion of the kidney

Under barbiturate anaesthesia the left renal artery was exposed and carefully isolated from the renal vein. Siliconized polyvinyl tubing was threaded through a Büchler peristaltic pump; one end was inserted into the carotid artery and the other was placed in the renal artery of the same rat. Before cannulation the tubing was primed with 0·6 ml of heparinized (1,000 units/ml) whole blood obtained from a rat having bilateral renal vascular ligation. Systemic arterial pressure was recorded from a femoral artery. Blood flow through the renal artery was 0·2 ml/min and the perfusion pressure was continuously recorded from a T connexion in the renal artery cannula. Artificial respiration was maintained in spinal sectioned preparations. Constant flow perfusion was evaluated under the following conditions: (1) A normal kidney (no ischaemia) was perfused at a constant flow in intact animals and 30 min after a C_1–C_2 spinal section in other animals; (2) The response of an ischaemic kidney (4 h) to constant flow perfusion was evaluated in intact rats and in rats in which the spinal cord had been transected between C_1 and C_2.

Presentation of results

Animals subjected to only the basic ischaemic kidney procedure were regarded as the control group. All other groups of rats that received drugs or underwent further surgical manipulations were compared with this group. The ischaemic kidney pressor response was compared among the various groups by Student's *t* test. Pre- and post-treatment basal blood pressures in the same animals were compared by the paired *t* test. Probabilities of 5% or less were regarded as statistically significant.

Results

Blood pressure response following re-establishment of circulation to the ischaemic kidney

The typical blood pressure response (control) after removal of the clamp from the renal artery was an elevation of 52 ± 4·7 mmHg with a rapid onset (<0·5 min)

TABLE 2. *Modification of the pressor response after re-establishing circulation to the ischaemic kidney*

Treatment	Blood pressure, mmHg ± S.E. Pre-treatment	Post-treatment	Maximum increase	*n*
Control	110 ± 4·5	110 ± 4·5	52 ± 4·7	7
Ligation of contralateral kidney	—	114 ± 8·7	45 ± 4·0	6
Cross perfusion:				
Donor (ischaemic kidney)	—	99 ± 6·4	†	4
Recipient	—	98 ± 13·1	49 ± 15·9	4
Phenol-renal stripping	—	92 ± 5·2*	52 ± 4·7	5
Phenol-renal stripping + papaverine	104 ± 7·1	99 ± 4·3	60 ± 4·3	5
Ureter ligation	—	98 ± 5·8	68 ± 4·0*	5

* $P<0·05$ compared with control. † Following clamp release blood pressure gradually declined 8 to 15 mmHg in these animals.

reaching a peak in about 4 min (Table 2). The pulse pressure usually widened and 50% recovery from the maximum amplitude of the response took about 20 minutes. Ligation of the contralateral renal vasculature just prior to clamp release did not modify ($P>0.05$) the blood pressure response indicating that the contralateral kidney is not required (Table 2). The cross-perfusion experiments in which the blood from the ischaemic kidney was transfused to a recipient rat demonstrated that the pressor response was due to a humoral material from the ischaemic kidney. Typical pressor responses appeared in the recipient rats but not in the donor animals (Table 2). There was a time lag of about 3 min before the onset of the response in the recipient animal which coincided with the time required for the transfused blood to reach the jugular vein of the recipient rat.

Effects of neural sections on the ischaemic kidney pressor response

In agreement with Hayden & Targett (1971a) spinal section between C_1 and C_2 significantly ($P<0.01$) inhibited the ischaemic kidney pressor response (Table 3). Similarly, pithing of the spinal cord completely abolished ($P<0.01$) the blood pressure rise after removing the renal arterial clamp (Table 3). Neither midthalamic

TABLE 3. *Effect of nerve sections on the elevation in blood pressure after re-establishment of circulation to the ischaemic kidney*

Level of nerve section	Blood pressure, mmHg ± S.E. Basal	Maximum increase	*n*
Control	110± 4·5	52±4·7	7
Midthalamic	130± 3·6†	60±7·7	6
Bulbar	80± 9·5*	63±8·1	5
C_1-C_2 spinal section	38± 4·8†	16±9·4†	6
Spinal pith	42±10·8†	0	4

* $P<0.05$ compared with control. † $P<0.01$ compared with control.

nor bulbar sectioning altered the ischaemic kidney pressor response ($P>0.05$, Table 3). Gross dissection showed that midthalamic sectioning left mesencephalic, and in most cases hypothalamic, cardiovascular pathways intact. Bulbar sectioning left the medulla and at least the caudal third of the pons intact. These results indidate that a neural requirement for the response may be located at high spinal or low bulbar levels.

Effects of pharmacological agents on the ischaemic kidney pressor response

The effects of all drugs studied are summarized in Table 4. Two classical α-adrenoceptor blocking drugs, phentolamine and phenoxybenzamine, failed to antagonize the ischaemic kidney pressor response. In fact, phentolamine treatment potentiated the response ($P<0.05$). This might be related to the lower basal blood pressure. Two β-adrenoceptor blocking drugs, propranolol and LB-46 ((±)-4-(2-hydroxy-3-isopropylaminopropoxy)-indole), were also ineffective as antagonists of renin release from the ischaemic kidney. Blockade of muscarinic receptors and ganglia with atropine-hexamethonium treatment was similarly ineffective. Three adrenergic neurone inhibiting drugs, reserpine, guanethidine, and 6-hydroxydopamine, were tested but only reserpine inhibited ($P<0.01$) the ischaemic kidney pressor response. The reason for reserpine's effectiveness is unknown. A 5-hydroxy-

TABLE 4. *Effect of pharmacological antagonists on the blood pressure elevation after re-establishing circulation to the ischaemic kidney*

Treatment*	Blood pressure, mmHg±S.E. Pre-treatment	Post-treatment	Maximum increase	*n*
Control (untreated)	110±4·5	110±4·5	52± 4·7	7
Phentolamine	116±4·4	82±7·3‡§	74± 6·1†	7
Phenoxybenzamine	—	117±7·7	42± 3·2	6
Propranolol	115±5·8	111±3·7	47± 7·4	6
LB-46	97±4·4	95±5·1	58± 8·6	5
Atropine+hexamethonium	115±8·2	101±6·2	52±10·2	7
Reserpine	—	83±5·2‡	21± 6·9‡	9
6-Hydroxydopamine	—	97±3·8	46±10·0	6
Guanethidine	—	108±5·7	50± 7·3	7
Diphenhydramine	105±7·2	117±5·3	42± 9·3	6
Methysergide	119±6·7	112±5·1	48± 7·0	8

* See Table 1 for details of drug treatment. † $P<0·05$ compared with control. ‡ $P<0·01$ compared with control. § $P<0·05$ pre-treatment vs. post-treatment.

tryptamine antagonist (methysergide) and an antihistamine (diphenhydramine) had no effect on the pressor response.

Pharmacological agonists were administered approximately one hour after release of the renal artery clamp to evaluate the effectiveness of the blocking compounds. Inhibition of blood pressure changes induced by specific agonists indicated that all of the antagonists studied exerted their characteristic effects. The dosage regimen of 6-hydroxydopamine used reduces rat kidney noradrenaline levels from 238±18 to 62±8 ng/g±S.E. ($n=10$ per group). The pharmacological data appear to suggest that the sympathetic nervous system is not required for renin release from the ischaemic kidney.

Effect of ureter ligation and papaverine treatment

It is probable that the ischaemic kidney was nonfiltering. However, to further ensure that this was the case, the ischaemic kidney preparation was studied after ureter ligation. This procedure increased the pressor response ($P<0·05$, Table 2) after release of the clamp on the renal artery. The precise reason for this increase is unknown, but it is unlikely that renin was released by the macula densa mechanism (Witty, Davis, Johnson & Prewitt, 1971).

Witty *et al.* (1971) found that papaverine infusion into the renal artery blocks renin release from the denervated nonfiltering dog kidney in response to haemorrhage thereby suggesting that papaverine inhibits the baroreceptor mechanism for control of renin secretion. Although this effect of papaverine has not been systematically demonstrated in rats we administered large doses of the drug in an attempt to uncover possible involvement of the baroreceptor mechanism of renin release. Physical stripping of the adventitia and phenol application to the main vessels and pedicle areas of the ischaemic kidney to produce acute denervation did not change the typical pressor response. Papaverine administration was also ineffective (Table 2). These experiments provide suggestive evidence that baroreceptor-induced renin release may not explain the ischaemic kidney pressor response.

Constant flow perfusion of ischaemic kidneys

Collectively, the above data suggest that neither sympathetic innervation, nor renal baroreceptor stimulation, nor macula densa activation is sufficient to explain

TABLE 5. *Effect of constant flow renal perfusion in intact and cervical spinal-sectioned rats*

Preparation	Blood pressure, mmHg ± S.E. Basal	Increase	Perfusion pressure (mmHg) ± S.E.	*n*
Ischaemic kidney	126 ± 3·2*	37 ± 1·9†	149 ± 23·0	7
Ischaemic kidney + C_1-C_2 section	47 ± 4·4‡	80 ± 9·0‡§	134 ± 20·7	7
Non-ischaemic kidney	107 ± 6·4	8 ± 2·5	122 ± 21·5	6
Non-ischaemic kidney + C_1-C_2 section	49 ± 8·0†	9 ± 2·1	124 ± 16·5	6

* Compared with non-ischaemic kidney, $P<0·05$. † Compared with non-ischaemic kidney, $P<0·001$. ‡ Compared with ischaemic kidney, $P<0·001$. § Compared with non-ischaemic kidney + C_1-C_2 section, $P<0·001$.

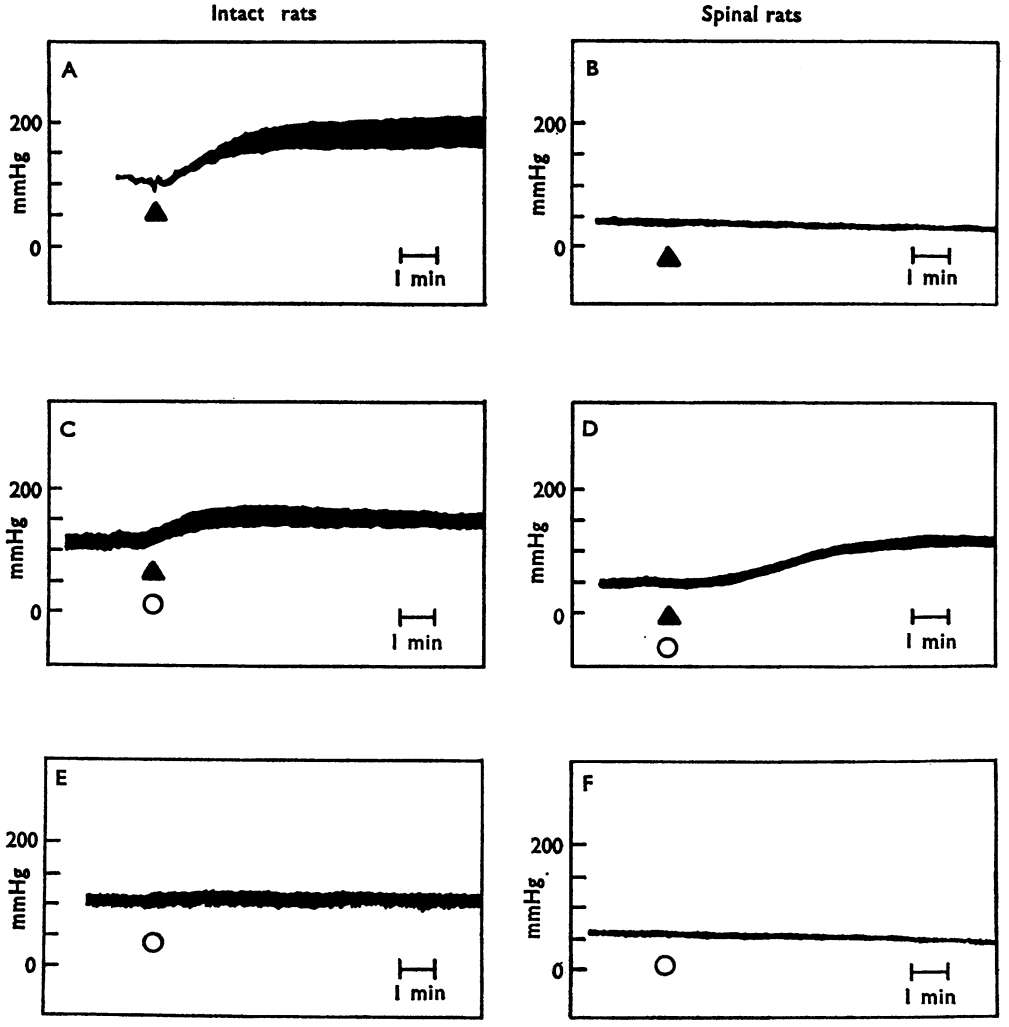

FIG. 1. Typical blood pressure responses in intact and spinal-sectioned rats with and without constant flow perfusion of the ischaemic kidney. ▲=Release of renal arterial clamp; ○= start constant flow perfusion of kidney. Panels A and B, blood pressure response after renal arterial clamp removal in an intact (A) and spinal (B) rat. Panels C and D, same as panels A and B, respectively, except that the ischaemic kidney was constant flow perfused when the renal arterial clamp was removed. Panels E and F, same as panels C and D, respectively, except that the perfused kidney was not ischaemic. Compare with the ischaemic kidney response in panels C and D.

the ischaemic kidney pressor response. The ischaemic kidney preparation was tested under conditions of constant flow perfusion to evaluate whether the effects of spinal section or pithing were secondary to poor renal blood flow. The results of these experiments are summarized in Table 5. It may be seen that constant flow perfusion of non-ischaemic kidneys in intact or spinal sectioned rats was not associated with a pressor response. However, ischaemic kidneys from both intact and spinal sectioned rats caused a pressor response when perfusion was initiated. The spinal sectioned rats, under these conditions, displayed an enhanced ($P<0.01$) pressor response compared with the intact rats. This could be due to the very low basal blood pressure. The perfusion pressures of all groups of rats were similar (Table 5). Typical blood pressure tracings of intact and spinal sectioned rats with and without constant flow perfusion are shown in Figure 1. The appearance of pressor activity in spinal sectioned rats during constant flow perfusion of the ischaemic kidney suggests that any neural requirement for renin release from the ischaemic kidney is related to maintenance of adequate renal blood flow.

Discussion

Renin release from the ischaemic kidney was thought to be the result of its leakage from cells into the renal venous blood after circulation is re-established (Taquini *et al.*, 1964). The data of Hayden & Targett (1971a, b) suggest that renin release from the ischaemic kidney is more complicated than a simple 'washout' phenomenon. These investigators found that spinal section or stellate ganglionectomy blocked the rise in plasma renin activity and the concomitant blood pressure elevation after re-establishing circulation to the ischaemic rat kidney. According to Hayden & Targett (1971a) '. . . it is clear that the neural release of renin is inhibited; and although some renin may be released by either a renal baroreceptor or a macula densa mechanism it is insufficient to form enough angiotensin to cause a peripherally mediated pressor response.'

The nervous system could be required for renin release from the ischaemic kidney by: (1) increasing renin efflux from juxtaglomerular cells; (2) maintaining adequate blood flow for renin 'washout' into the general circulation; or (3) producing a combination of these effects. Hayden & Targett (1971a) evaluated circulation through the ischaemic kidney by the injection of 100 μg (0·1 ml) of adrenaline tartrate into the kidney pulp. For several reasons the adequacy of this test is difficult to assess. Firstly, adrenaline itself could alter intrarenal distribution of blood. Secondly, the adrenaline test might not reflect the possibility that circulation through the ischaemic kidney was very slow because of a low perfusion pressure. The latter criticism is important since minimal circulation through an ischaemic kidney might deliver renin into the blood stream at nonpressor or minimally pressor rates, and thus it would appear that the ischaemic kidney pressor response was blocked or inhibited.

In the present report we have confirmed Hayden & Targett's (1971a) observation that high spinal section inhibits the ischaemic kidney pressor response. We have also extended this finding to include pithed rats. Since we were unable to demonstrate a renin-releasing role for the sympathetic nervous system by a variety of pharmacological antagonists it is unlikely that sympathetic innervation is required for the ischaemic kidney pressor response. We were also unable to demonstrate

that macula densa or baroreceptor mechanisms are required for renin release from the ischaemic kidney. A pressor response was consistently obtained by constant flow perfusion of ischaemic kidneys in spinal sectioned rats. Therefore, it is probable that renin release from the ischaemic kidney is independent of the nervous system, provided adequate blood flow through the kidney is maintained. The data obtained in this investigation support the original conclusions of Taquini *et al.* (1964) that renin release after re-establishing circulation to the ischaemic kidney is a 'washout' phenomenon.

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