It is suggested that the reduction in filtration rate, urine flow and effective renal blood flow in poisoned pullets resulted either from a rapid redistribution of arterial blood away from the kidneys, or from renal arteriolar constriction which could result in a reduction in glomerular capillary pressure. However, the mechanisms by which the observed changes in renal homeostasis are effected in the poisoned birds and alleviated in the chelate-primed birds remain largely unresolved.

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Effect of bilateral nephrectomy on the recovery of blood pressure after acute hemorrhage in rats: role of renin-angiotensin system

L. F. O. Obika

Department of Physiology and Biochemistry, Faculty of Health Sciences, University of Ilorin, Ilorin (Nigeria), 25 June 1985

Summary. The effect of bilateral nephrectomy, and administration of an inhibitor of angiotensin converting enzyme, on the recovery of arterial blood pressure after hemorrhage (loss of 1% of b.wt), was studied in male Sprague-Dawley rats. Neither manœuver significantly affected the recovery of blood pressure within the first 10 min after hemorrhage. Thereafter, the recovery of the blood pressure was markedly suppressed. The study suggests that the initial recovery of blood pressure is unrelated to the kidneys, but the later one requires their presence and depends on the activity of the renin-angiotensin system.
Key words. Hemorrhage; blood pressure; renin-angiotensin system.

The fall in blood pressure following a loss of blood from the vascular system initiates rapid nervous and hormonal compensatory responses. Many investigators have described activation of the renin-angiotensin system in response to acute hemorrhage[1,2]. But the role of the kidney as a homeostatic organ for blood pressure maintenance during hemorrhage has not been fully described. The results of Regoli[3] suggest that nephrectomy exerts little influence on the time course of blood pressure response after hemorrhage. Sapirstein et al.[4] postulated that the renin-angiotensin system is the renal compensatory mechanism for maintenance of blood pressure in response to hemorrhagic hypotension. Zerbe et al.[5] showed that blockade of angiotensin II formation is accompanied by a blunted recovery of blood pressure after hemorrhage.

Earlier evidence[6,7], however, suggests that angiotensin may not be the pressor material released by the activation of the kidney, and in fact that renal factors other than the renin-angiotensin system may be involved in the compensatory response to hemorrhagic hypotension.

The present experiments were therefore designed to re-examine the role of the kidney in the recovery of blood pressure after hemorrhage in bilaterally nephrectomized rats and in rats that had a bolus injection of the converting-enzyme inhibitor, captopril, before the hemorrhage.

Material and methods. Male Sprague-Dawley rats weighing between 180 and 280 g were used. They were housed in the laboratory and allowed free access to standard laboratory rat pellets and water.

Experimental protocol. Anesthesia was induced with sodium pentobarbital (50 mg/kg, i.p.; Abbott Laboratories, Ill., USA). The animals were reweighed after the induction of anesthesia. Polyethylene catheters filled with heparin solution (100 units per 100 ml of saline) were inserted into the right carotid artery and the left external jugular vein. The rats were subsequently divided into four groups as shown in the table. Bilateral nephrectomy was performed through a midline incision. Non-nephrectomized rats were sham-operated.

The animals were allowed to equilibrate for 45 min, after which the mean blood pressure was recorded (pre-hemorrhage blood pressure) on a Gilson Polygraph Model 5/6H with a Stathem P23ID transducer via the carotid artery. Group 4 rats received a bolus injection of 1 mg/kg captopril (Squibb Inst., Princeton, NJ, USA) via the jugular vein after the equilibration period. 0.1 ml 5% dextrose was used to flush the jugular vein catheter after injection. In this group, the pre-hemorrhage blood pressure was recorded 10 min after the captopril injection and this was followed by the hemorrhage. Hemorrhage in all rats was carried out within 3–5 min via the carotid artery after the equilibration period of 45 min, and recording of the pre-hemorrhage blood pressure. The blood pressure was thereafter read at intervals of 5, 10, 20, 30, 40 and 60 min and recorded continuously for 60 min after hemorrhage.

Results are expressed as the mean±SEM and compared by Student's t-test or analysis of variance using a between and within design for repeated measures. A value of 0.05 or less was considered significant.

Results. The results are shown in the table and the figure.

a) Effect of nephrectomy. The blood pressure in nephrectomized

Baseline data on the role of the kidneys in the recovery of blood pressure after hemorrhage

Group	N	Weight (g)	Blood pressure before hemorrhage (mm Hg)	Volume of blood removed (ml)
1) Nephrectomy No hemorrhage	8	229 ± 5	98 ± 3	–
2) Nephrectomy Hemorrhage	7	233 ± 11	97 ± 3	2.4 ± 0.1
3) No nephrectomy Hemorrhage	7	239 ± 6	108 ± 5	2.5 ± 0.1
4) No nephrectomy Captopril, 1 mg/kg	7	229 ± 11	106 ± 6*	2.4 ± 0.2

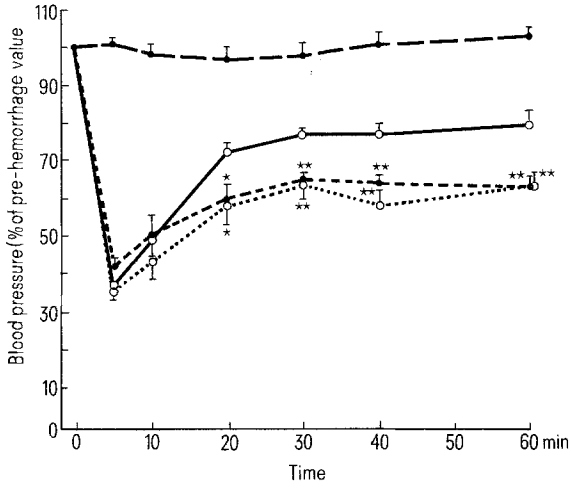
*Blood pressure value 10 min after captopril injection. Values are means ± SEM.

rats without hemorrhage remained steady throughout the 60 min of study (fig.). When compared to non-nephrectomized rats (groups 3 and 4; 116±4 mm Hg) there was a statistically significant difference ($t = 3.76$; $p < 0.001$) in the baseline blood pressure of the nephrectomized rats (groups 1 and 2; 97±2 mm Hg). Thus acute nephrectomy lowered the basal blood pressure of these rats by about 19 mm Hg.

b) Effect of hemorrhage in nephrectomized rats. There was no difference in the pre-hemorrhage blood pressure between the two groups of nephrectomized rats (table, groups 1 vs 2). Hemorrhage, however, decreased the blood pressure by about 45% within 10 min. The blood pressure recovered to about 60% of the control value in the next 10 min and remained at this level until the end of the experiment (fig.). When compared to non-nephrectomized but hemorrhaged rats (group 3), the nephrectomized rats' blood pressure after hemorrhage was lower from 20 min after the hemorrhage until the termination of the experiment.

c) Effect of hemorrhage in non-nephrectomized rats. Hemorrhage caused a fall of blood pressure to about 45% of the pre-hemorrhage value within 10 min. 20 min after hemorrhage, the blood pressure had recovered to about 70% of the pre-hemorrhage value and remained at about that value until the end of the experiment (fig.). When compared to the nephrectomized rats, these observations suggest that 1) the kidney does not play a role in the recovery of blood pressure immediately after hemorrhage, 2) the recovery of blood pressure is blunted in nephrectomized rats 20 min and up to 60 min after hemorrhage.

d) Effect of hemorrhage in captopril-treated non-nephrectomized rats. There was no statistically significant difference in the pre-hemorrhage blood pressure in the non-nephrectomized rats (groups 3 vs 4). In group 4, 10 min after the injection of captopril the blood pressure fell by 10.8±3.2 mm Hg ($p < 0.01$; paired t-test). The recovery of blood pressure within the first 10 min after hemorrhage in non-nephrectomized, but captopril-treated rats was similar to the hemorrhage rats in groups 2 and 3; thereafter, captopril-treated rats showed a suppressed recovery of the blood pressure similar to nephrectomized rats. These observations suggest that the blockade of the renin-angiotensin system blunts the recovery of blood pressure, a response that is not different from that of nephrectomized rats.



Effect of bilateral nephrectomy and ACE inhibition on the spontaneous recovery of blood pressure after hemorrhage. Values are means±SEM.
●——●, Bilateral nephrectomy, no hemorrhage, n = 8;
●----●, bilateral nephrectomy, hemorrhage, n = 7;
○——○, no nephrectomy, hemorrhage, n = 7;
○ · · · · · ○, no nephrectomy, ACE inhibition, hemorrhage, n = 7.
*$p < 0.05$; **$p < 0.01$ significantly different from no nephrectomy but hemorrhage group (ACE = angiotensin converting enzyme).

Discussion. Hemorrhage is known to activate the renin-angiotensin system[1,8], which may be important for the recovery of blood pressure after hemorrhage[4,9]. The principal pressor system of the kidney is the renin-angiotensin system[10]. The renin activity in the blood of rats, dogs, and cats originates mainly from the kidneys[10] and falls to very low levels after nephrectomy[11] or captopril treatment[12]; but there are still conflicting views about the role of the kidney in the homeostasis of blood pressure after hemorrhage[3,13,14]. In the cat, Fuerstein et al.[14] showed that the increase in the plasma renin concentration and the recovery of blood pressure after hemorrhage were abolished by bilateral nephrectomy, albeit 40 min after the hemorrhage. In addition, Fuerstein and Cohen[9] and Zerbe et al.[5] demonstrated that the renin-angiotensin system is a vital component of the compensatory mechanism responsible for blood pressure recovery after hemorrhage. The results reported here show that the kidney is important only in the later recovery of blood pressure after hemorrhage.

The orally active compound captopril has been shown to be an effective and potent inhibitor of the converting enzyme[12]. In the study reported here, a bolus injection of captopril, 10 min before hemorrhage, suppressed the recovery of blood pressure after hemorrhage. This is in agreement with the result of Zerbe et al.[5]. Taken together, these observations are suggestive of a role of the kidneys via the renin-angiotensin system in blood pressure homeostasis after hemorrhage. However, this role seems not to be important immediately after hemorrhage, since the blood pressures of non-nephrectomized and nephrectomized rats were similar up to 10 min after hemorrhage. Perhaps a different mechanism is operative during this period. This has been suggested by the work of Laycock et al.[15] who showed that in the rat, vasopressin is important in the recovery of blood pressure immediately after hemorrhage. Their observation has been supported by other investigators[16,17].

Another important observation in this study is the fact that hemorrhage induced the same decrease of blood pressure within 10 min in rats so treated. Zerbe et al.[5] showed that rats treated with captopril had basal and post hemorrhage blood pressures comparable with those of the control rats. But, in the dog, Hamilton and Cohen[13] observed that the blood pressure of nephrectomized dogs fell far more than that of normal dogs when an equal amount of blood was removed from each group. This is in contrast to the result of the present study, perhaps because of the species difference. However, DuCharme and Beck[6] noted from their studies that the volumes of blood removed to maintain a hypotensive pressure in nephrectomized and sham-operated dogs were not different within the initial 2 min after hemorrhage. They concluded that the kidneys do not contribute to the maintenance of blood pressure.

In conclusion, this study shows the participation of the kidneys, and suggests an involvement of the renin-angiotensin system in the recovery of blood pressure after moderate hemorrhage in the rat.

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Afferent fibers from the septum terminate on gamma-aminobutyric acid (GABA-) interneurons and granule cells in the area dentata of the rat

W.K. Schwerdtfeger

Max-Planck-Institut für Hirnforschung, Deutschordenstrasse 46, D-6000 Frankfurt am Main 71 (Federal Republic of Germany), *5 July 1985*

Summary. Interneurons in the area dentata of the rat were immunostained with an antibody to gamma-aminobutyric acid. After septal lesions, degenerating terminals were found in asymmetric synaptic contact with granule cell somata and dendritic elements of immunoreactive and nonreactive cells in the supragranular part of the molecular layer.
Key words. Hippocampus; area dentata; GABA; interneurons; septal afferents.

The septo-hippocampal projection has been reported to terminate in virtually all areas of the hippocampus. In the area dentata, septal fibers end in the supragranular part of the molecular layer and in the hilus[2]. A greater part of this projection is cholinergic[3,4], but probably GABAergic fibers[5] and substance P-fibers[6] are also included. The cholinergic septal fibers are believed to act in an excitatory way on the granular and pyramidal cells of the hippocampus in a pacemaker action to generate the atropine-sensitive (type 2) type of the theta or rhythmic slow activity[7] which may be correlated with sensory processing in the hippocampus[8]. However, it is not yet clear whether the targets of the septo-hippocampal projections are only projection neurons (pyramidal and granular cells), or also interneurons, which is of interest in view of the proposition that part of the effect of acetylcholine is mediated by interneurons[9,10]. In the present study, immunocytochemical investigation of GABA-interneurons was performed in the area dentata of the rat in combination with degeneration studies after midseptal lesions.

Materials and methods. Under deep Nembutal anesthesia, lesions were made in the medial septum of adult Wistar rats by electrocoagulation with anodal current (5 mA, 20 s) applied through the tip of an insulated stainless steel electrode. Electrodes were positioned in the midsagittal plane, thus avoiding the neocortex. Inevitably, electrodes penetrated the corpus callosum. However, this additional damage should not constitute a problem in this case; so far, we do not know of any hippocampal afferent pathway passing via the corpus callosum. Two days later, the animals were injected with 50 µg colchicine in 5 µl 0.9% saline[11] in the neocortex overlying the hippocampus to inhibit fast intraaxonal transport[12]. The injections were not given directly into the hippocampus, to avoid additional lesioning. On the following day the animals were sacrificed by transcardial perfusion with 50 ml saline followed by fixative consisting of 4% paraformaldehyde and 0.09% glutaraldehyde, in 0.1 M phosphate buffer (pH 7.3). Hippocampi were removed, cut into small blocks and left for 3 h in the same fixative. After overnight immersion in phosphate buffer with 30% sucrose, blocks were rapidly frozen by plunging into liquid nitrogen[13], thawed by returning them to 30% sucrose, and cut into 40 µm thick sections on a vibratome. Sections were stored in test tubes containing phosphate buffered saline (PBS).

To aid penetration of the antibody into the sections, all the following reactions were made with the sections floating free. Prior to the antibody incubation, all sections were immersed in a solution of 3% H_2O_2 in methanol to inactivate endogenous peroxidases. Sections were incubated overnight at 4 °C with an antibody to gamma-aminobutyric acid (GABA) raised in rabbits (Immunonuclear Corp., USA) diluted 1:2000 in PBS containing 1% normal goat serum. Control sections incubated in nonimmune serum later showed no reaction product. The avidin-biotin-complex (ABC)-method[14] was used for the visualization of the antibody-antigen complex. In short, sections were washed in 0.1 M phosphate-buffered saline (PBS) and incubated for 1 h with biotin-coupled goat anti-rabbit IgG (Vector Labs., USA), washed again and incubated for another hour with avidin and biotinylated peroxidase (Vector Labs., USA). After washing, the sections were reacted with a freshly filtered solution of 0.05% 3,3'-diaminobenzidene (Sigma) and 0.04% H_2O_2 in PBS. Sections were postfixed in OsO_4, processed for electron microscopy and embedded in a thin layer of Durcupan between two transparent plastic foils. After polymerization of the medium, the preparations were checked. Cells with visible processes were cut out and glued onto plastic blocks for photographing (fig. 1) and final ultra-thin sectioning.

Results. Septal lesions led to dark degeneration of axon terminals in the stratum moleculare, stratum granulare, and hilus of the area dentata. Terminals ranged in size between about 0.5 µm and 2 µm and had round or ovoid profiles. They contained spherical synaptic vesicles and formed asymmetric (Gray I) synaptic contacts within a narrow zone of about 50 µm in the supragranular part of the molecular layer, mostly with dendritic spines (fig. 2), to a lesser extent also with dendritic shafts; very few boutons were found in synaptic contact with somata of granule cells in the granular layer, which were identified by the regular shapes of their somata and nuclei.

Asymmetric contacts were easy to identify by a clear synaptic cleft and a prominent dark postsynaptic dense area. Some degenerating terminals formed contacts with more than one postsynaptic element (fig. 2a); sometimes more than one terminal was connected with the same dendritic spine (fig. 2b). Most of the degenerating boutons found were in contact with postsynaptic elements which lacked immunostaining. In contrast to the