Irreversible shock in rats after injection of microspheres into the renal artery[1]

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Summary. After injection of microspheres into both renal arteries of rats, an irreversible shock syndrome develops, resulting in death within 4–12 h. Ligation of both renal pedicles after injection of microspheres prevents the shock. It is presumed that kininogenases released from the kidneys participate in the pathogenesis of the shock syndrome.

Various groups have reported that the injection of microspheres into the kidneys of rats induces a sustained elevation of blood pressure[2,3]. The degree of hypertension depends on the amount and the size of the microspheres. In contrast to these chronic experiments, the acute effects elicited by the injection of microspheres into the renal artery have hardly been investigated so far. In former studies with the isolated perfused rat kidney, a transient release of renin and an impaired renal function were found after 0.5 mg of microspheres had been injected into the perfusion fluid[4].

In the experiments presented here, the immediate and delayed effects of intrarenal injections of various amounts of microspheres into one or both renal arteries have been investigated.

Material and methods. Male Sprague-Dawley rats of a body weight between 200 and 300 g were used. The animals were anaesthetized with thiobutabarbital (Inactin®) in a dose of 100 mg/kg, half of the dose being administered i.p., the other half s.c. The blood pressure was recorded continuously by a polyethylene catheter placed into the left carotid artery and connected to a pressure transducer (Statham Pb 23 Dc). Similarly, the central venous pressure was monitored in the right atrium by a strain gauge Statham Pb 23 BB. The heart rate was taken from rapid blood-pressure recordings.

Non-radioactive tracer microspheres (3M brand) of various sizes – 15, 25, 35, 50 and 80 μm – were suspended in a 3.5% gelatine solution (Haemaccel®), and a homogenous suspension was obtained by ultrasonic exposure for 3–4 min. The suspension, ready for injection, contained either 20 mg or 50 mg per ml. Since preliminary studies did not indicate that the results depended on the size of the microspheres, microspheres of 50 μm were used in all experiments reported here.

Packed cell volume (PCF), serum urea and plasma potassium concentrations of groups B, C and D immediately after the operation (0) and 2, 4 and 8 h later. All figures are means ± SEM (u = 6 each)

	0	2	4	8 h
Group B				
PCV (percent)	42.5 ±0.38	56.12±2.62	54.9 ±2.0	48.6 ±1.38
Urea (mmoles/l)	5.22±0.75	10.22±1.00	15.0 ±1.50	25.00±1.75
Potassium (mEq/l)	3.90±0.25	6.0 ±0.6	5.5 ±0.55	5.50±0.80
Group C				
PCV (percent)	42.75±0.38	43.00±0.75	44.4 ±0.38	45.25±1.0
Urea (mmoles/l)	5.75±0.75	10.22±0.75	11.50±1.00	25.5 ±1.0
Potassium (mEq/l)	4.00±0.25	5.85±0.30	7.00±0.60	7.5 ±0.55
Group D				
PCV (percent)	42.62±0.38	42.38±0.63	42.62±0.63	48.0 ±1.62
Urea (mmoles/l)	5.5 ±0.50	8.75±0.75	10.0 ±1.25	28.0 ±0.75
Potassium (mEq/l)	4.1 ±0.25	4.45±0.20	5.0 ±0.40	6.5 ±0.55

After cannulation of the abdominal aorta above the iliac bifurcation, the tip of the cannula was placed just below the origin of the renal arteries. Before the injection of the microsphere suspension into one or both renal arteries, the aorta, distal and proximal to the renal arteries, was transiently clamped so as to direct the total injection volume of 0.5 ml into each kidney. In the experiments in which the microspheres were injected into only one kidney, the artery of the contralateral kidney was also occluded during the injection period. After the injection, which lasted about 20 sec, the clips were removed from the aorta and from the other arteries (mesenteric) that had been transiently occluded. The cannula was removed from the aorta, and the site of insertion was closed by an autopolymerizing tissue stick (Histoacryl®). Subsequently, the wound was closed.

The rats in which the blood pressure was not recorded were operated in the same manner, but aether was used as anaesthetic. After the operation, the conscious rats were kept in individual cages for 2, 4 and 8 h. After these intervals, the animals were anaesthetized with aether, and blood was taken from the jugular vein for the determination of packed cell volume (PVC) and of serum concentrations of sodium, potassium and urea. The plasma concentration of angiotensin II was measured directly according to the method described previously[5]. Blood pH, pCO_2, base excess, standard bicarbonate and buffer base were measured by ASTRUP microequipment (AME 1).

The following experimental groups were studied:

Group A: 12 rats in which 0.5 ml containing 10 mg of microspheres was injected into each of the renal arteries;

Group B: 31 rats in which 0.5 ml containing 25 mg of microspheres was injected into each of the renal arteries;

Group C: 31 rats which received the same amount of microspheres as those of group B, but in which both renal pedicles were ligated after the injection;

Group D: 31 rats in which both renal pedicles were ligated, without injection of microspheres;

Group E: 6 rats in which 1 ml containing 50 mg of microspheres was injected into the left kidney, while the right renal artery was transiently occluded;

Group F: 5 rats in which 0.5 ml containing 25 mg of microspheres was injected into the left kidney. The ureter of the right kidney was ligated, and this kidney was removed 2–4 h later.

In 7 rats each of group B, C and D and in all rats of group F, the blood pressure was recorded continuously under thiobutabarbital anaesthesia.

1 These studies were supported in part by the Deutsche Forschungsgemeinschaft within the SFB 90, 'Cardiovasculäres System'.

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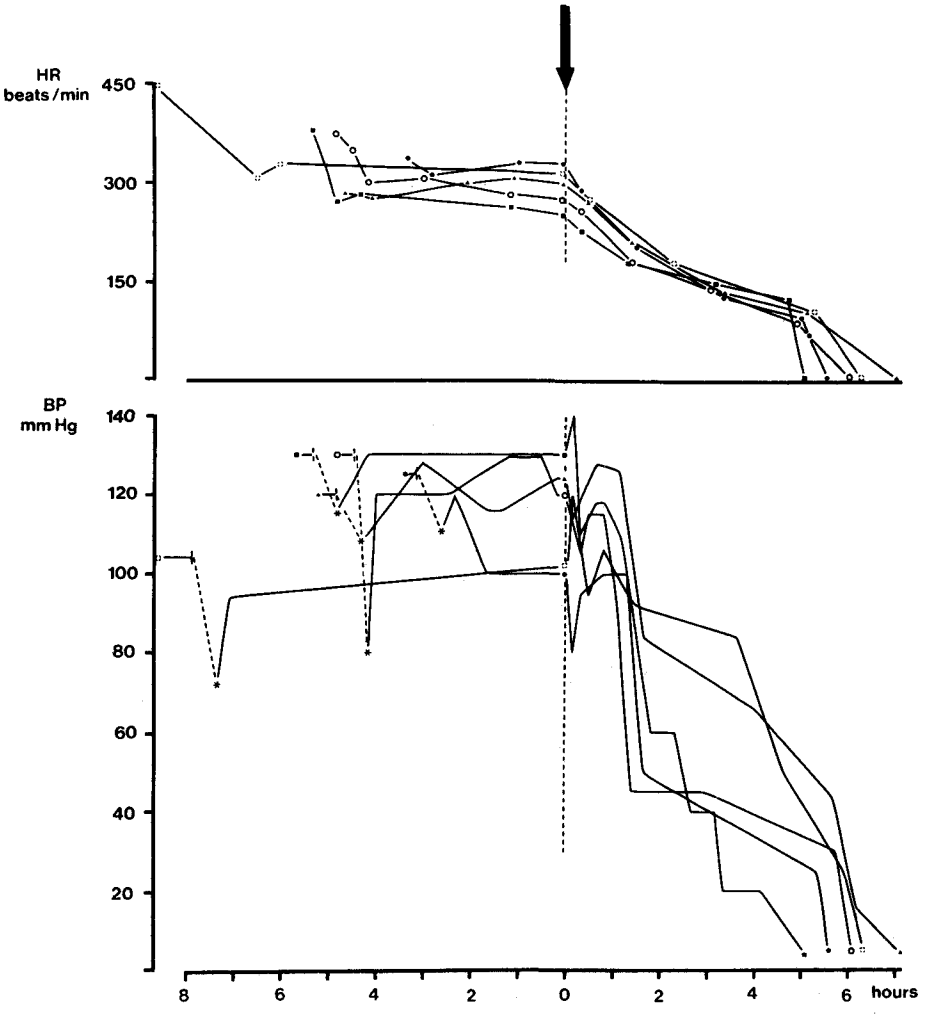
Results. After the injection of 10 mg of microspheres into one kidney, about half of the nephrons are obstructed, whereas the injection of 25 mg or 50 mg will result in a complete obstruction of the renocortical vascular bed. After the injection of 25 mg of microspheres into each kidney (group B), the blood pressure remained unchanged for about 1 h, but subsequently fell progressively. Death occurred 4–12 h after the embolization of the renal vascular bed. A similar fall in blood pressure was seen in the rats of group F, after the contralateral kidney, into which no microspheres had been injected, was removed (figure). All rats died within 5–7 h after removal of the non-obstructed kidney. In the rats of groups A, C and E, the injection of microspheres was not followed by a fall in blood pressure, and the same was the case after ligation of the 2 renal pedicles (group D). In the rats of group B, simultaneously with the reduction of the arterial pressure, the central venous pressure fell to about -4 cm H_2O, but it rose shortly before the animals died. In the 2 groups in which the blood pressure fell (B and F), a decrease in heart rate occurred, which, in most animals, began before the fall in blood pressure. In the other groups, with stable blood-pressure values, the heart rate did not change.

Other parameters: In groups B, C and D, serum urea rose similarly, up to values of 35 mmoles/l within about 6 h. Serum potassium was increased to 8 mEq/l in the rats of group C, but was less elevated in those of groups B and D (table). Metabolic acidosis was observed in all groups of rats, being most marked (pH 7.01) in group C, already 4 h after the injection of microspheres. The plasma angiotensin II concentration reached very high values in the animals of groups B and D, the excessive concentration of 12,000 pg/ml being measured in one animal and that of 1500 pg/ml in the remaining rats. In the rats of group C, the mean value was 500 pg/ml, which was about 4 times the control values (120–150 pg/ml). A marked rise in PCV from 43 to 56% was obtained in the rats of group B, already 2 h after the injection. In groups C and D, an increase of only 3 and 6% respectively was found after 8 h.

When the rats of group B were placed into metabolic cages, the maximum amount of urine produced during 8 h was 0.2 ml. At autopsy, a haemorrhagic peritoneal effusion of 1.5–2.5 ml was found, and also pleural exsudations were observed in some animals. In a few animals, into which Evans blue had been injected i.v. prior to the administration of the microspheres, the peritoneal exsudate was stained blue, as was the serum.

Discussion. From the experiments it is evident that embolic obstruction of the renal vascular bed is followed by the development of a shock-like state, as a consequence of which the animals die within 4–12 h. On the other hand, removal of both kidneys or ligature of the 2 renal pedicles results in uraemia, which is tolerated for 48–72 h. The shock is characterized by a slow fall in blood pressure and heart rate and an increased capillary permeability. No symptoms of shock are observed if the excretory



Heart rate (above) and blood pressure (below) after injection of 25 mg of microspheres into 1 kidney. The ureter of the contralateral kidney was ligated (group F). The arrow indicates the removal of the contralateral kidney.

function of the kidney is eliminated by ureter ligation. To a certain degree, the severity of the shock depends on the amount of the injected microspheres. After the injection of 10 mg into each kidney, the serum urea rose transiently, but no symptoms of circulatory failure occurred, and all animals survived for a period of more than 3 months. In contrast to this, the injection of 25 mg into each kidney always resulted in the rapid development of a severe shock-like state, and all animals succumbed within 12 h.

The shock-like state is prevented, if a contralateral, non-embolized kidney is present, independent of its excretory function. If the intact renal tissue is removed after several hours, a shock-like state still develops as a consequence of the preceding injection of microspheres into the other kidney.

It is suggested that the shock-like state after massive embolization is elicited by the release of a substance from the affected kidney. At first, it was supposed that renin might have been excessively released, and the high plasma concentration of angiotensin II seems to support such an assumption. However, the stimulation of renin secretion and the enhanced formation of angiotensin II should result in an increase in blood pressure. Furthermore, in studies with the isolated perfused kidney, only a transient release of renin for about 10 min was observed[4], but the amount of microspheres injected in those experiments was only a fraction ($^1/_{50}$) of that given to the rats in which a shock-like state developed. The high plasma concentration of angiotensin II is probably the consequence of a secondary activation of the renin-angiotensin system. The reduction in intravascular volume, caused by the increased capillary permeability, is the major stimulus for the renin-angiotensin system. In addition, renin might have been released also from the ischaemic renal tissue. A release of renal prostaglandins has also been considered, but when the rats were pretreated with indomethacin (2–3 mg/kg), the shock-like state could not be prevented. However, these experiments do not exclude a possible contribution of prostaglandins to the shock syndrome. Another possibility would be the release of kininogenases from the kidney and the formation of kinins, such as kallidin and bradykinin. If bradykinin was infused into rats, the blood pressure fell in relation to the dose, which ranged from 2.5 γ/kg/min to 125 γ/kg/min. The response to vasopressor agents, such as noradrenaline and angiotensin II, was reduced. A bolus injection of 50 γ bradykinin per rat (corresponding to 250 γ/kg) was followed by a decrease in blood pressure and heart rate, and the rats died within 10–40 min. Although these observations seem to support a causal role of bradykinin in the development of the shock syndrome after renal embolization, they do not explain the protective role of intact renal tissue. Furthermore, the amount of bradykinin that may be formed in the rats is limited by the amount of substrate present. Hence, convincing evidence is still lacking that the kininogenase-kinin system is the only factor involved in the pathogenesis of the observed syndrome. On the other hand, bradykinin has been claimed to be responsible for the shock that occurs after injection of trypsin and other proteolytic enzymes[6]. Studies with the protease inhibitor aprotinin could not be undertaken because of its high toxicity in rats.

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Hornet ventilation noise: Rhythm and energy content

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Summary. A new technique is used to measure the hornet's wing movement. It enables one to measure precisely the frequency of this movement, even in the presence of spurious 'noise'. This autocorrelation technique revealed that the hornet's wing stroke is divided into 3 smaller strokes when they are tired. The energy content in each stage can be measured using the autocorrelator.

Thermoregulation is a common phenomenon among Vespinae[1], who use both fanning with the wings and water transport to cool overheated nests[2–5]. In the case of the Oriental hornet, Vespa orientalis, several workers arrange themselves around the nest entrance, facing out in nests above ground or up in subterranean nests. At temperatures higher than optimal, these workers commence a constant and rapid fanning movement of their wings. The fanning workers are most frequently dispersed on the outside of the nest, at some distance from the entrance[6], but may also arrange themselves behind and around it, in the galleries between the entrance and the combs, and on the combs proper. According to Steiner[3], the adult wasps begin fanning their wings when the temperature of the surface of the brood comb reaches about 35 °C. Because the Oriental hornet builds its nests underground, the workers may commence fanning even at temperatures around 20 °C, provided the relative humidity is higher than 80%. This can be induced experimentally by moistening the soil around the nest, and, in the laboratory, by keeping the floor of the artificial breeding box (ABB) wet. In this case, one to several workers engage in thermoregulation, and their fanning activity can be observed and recorded over long periods without interruption.

In order to obtain accurate data on the fanning activity and the noise produced by it, we employed a technique well known in astronomy and related fields, but never before used in analyzing hornet ventilation noise. A series

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