

# Anaphylactoid reactions during hemodialysis in sheep are ACE inhibitor dose-dependent and mediated by bradykinin

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**Anaphylactoid reactions during hemodialysis in sheep are ACE inhibitor dose-dependent and mediated by bradykinin.** Anaphylactoid reactions (AR) have been attributed to the generation of bradykinin (BK) when AN69 membranes are used together with angiotensin converting enzyme (ACE) inhibitors during hemodialysis. However, conclusive evidence for the involvement of the BK as the mediator of these AR is still lacking. This study examined the degree of contact activation in an animal model caused by three PAN membranes—AN69, PAN DX, and SPAN—and the effects of different doses of the ACE inhibitor enalapril (ENA) and the BK B<sub>2</sub>-receptor antagonist icatibant on AR during hemodialysis. Six sheep were dialyzed for one hour with or without ENA pre-treatment using the different membranes in random order. Severe AR were observed only during hemodialysis with AN69 dialyzers together with ENA pre-treatment; the severity of AR increased with the ENA dose. Mild hypotension was noted during hemodialysis with AN69 without ACE inhibition and with PAN DX and 20 mg ENA. Compared to pre-dialysis values, maximum generation of BK after blood passage through the dialyzer was found at five minutes: 73-fold (AN69 without ENA), 161-fold (AN69 with 10 mg ENA), 97-fold (AN69 with 20 mg ENA), 108-fold (AN69 with 30 mg ENA), 154-fold (AN69 with 30 mg ENA and 0.1 mg/kg icatibant), 18-fold (PAN DX without ENA), and 42-fold (PAN DX with 20 mg ENA). Elevated BK levels in arterial blood were detected during hemodialysis with AN69 membranes even without ACE inhibition (2.5-fold); pre-treatment with 20 mg ENA further increased arterial BK concentrations (4-fold). Furthermore, a marked decline of prekallikrein and high molecular weight kininogen concentrations was noted for both AN69 and PAN DX membranes. Anaphylactoid reactions during hemodialysis were completely prevented by icatibant even after pre-treatment with ENA and in the presence of high BK concentrations. Concentrations of prekallikrein, high molecular weight kininogen, and BK remained unchanged and no AR were observed during hemodialysis with SPAN and pre-treatment with 20 mg ENA. Our findings confirm that AR during hemodialysis with the negatively charged AN69 membrane are mediated by BK, since they can be prevented by the BK B<sub>2</sub>-receptor antagonist icatibant.

Anaphylactoid reactions during hemodialysis with high-flux polyacrylonitrile (AN69) dialyzers in patients receiving angioten-

sin converting enzyme (ACE) inhibitors were first observed in 1990 [1, 2]. Since then, about 50 cases have been reported [3–7]. Apart from a possible involvement of bacterial endotoxins, contact activation initiating bradykinin generation together with a reduction of kininase II (= ACE; the key enzyme involved in kinin degradation) activity resulting from pre-treatment with ACE inhibitors have been implicated as pathogenetic factors in these reactions [1, 2]. Evidence supporting the role of bradykinin in anaphylactoid reactions has come from *in vitro* and *in vivo* studies showing that incubation of AN69 membranes with human plasma led to bradykinin generation [8, 9]. A dose-dependent addition of an ACE inhibitor resulted in a further increase and stability of bradykinin levels [8, 9]. The association of AN69 membrane usage and bradykinin release in blood after passage through the dialyzer was confirmed in an animal study with sheep; pre-treatment with the ACE inhibitor captopril resulted in even higher bradykinin levels and severe anaphylactoid reactions [10]. These findings were in accordance with clinical data of patients treated with ACE inhibitors showing high bradykinin levels at the onset of anaphylactoid reactions during hemodialysis with AN69 dialyzers [11].

To date, confirmatory evidence of the involvement of bradykinin as the mediator of these anaphylactoid reactions is still lacking. The present study was performed to investigate the extent of contact activation induced by three different polyacrylonitrile membranes—AN69, PAN DX, and SPAN—and their ability to evoke anaphylactoid reactions in an animal model. To add further proof to the “bradykinin hypothesis,” we wished to examine the effects of different ACE inhibitor doses and the effects of the selective bradykinin B<sub>2</sub>-receptor antagonist icatibant (Hoe 140) on anaphylactoid reactions and bradykinin generation during hemodialysis with the AN69 membrane.

## METHODS

### Animal model

An animal model using sheep was chosen for the studies. Sheep are very suitable for hemodialysis experiments since their body mass and blood circulation parameters are comparable to that of humans, as well as providing good vascular access [10].

Six female blackhead sheep, weighing between 51.4 and 99.4 kg, were hemodialyzed in a random order. Vascular access was achieved with a double lumen hemodialysis catheter inserted into

**Key words:** bradykinin, icatibant, ACE inhibition, membrane biocompatibility, polyacrylonitrile membranes, AN69 membrane.

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a jugular vein. A blood flow rate of 200 ml/min was selected. Acetate dialysate (concentrate HDY 76<sup>®</sup>, calcium concentration 1.75 mmol/liter; B. Braun Melsungen AG, Germany) and a Braun hemodialysis machine (HD Secura<sup>®</sup>) was used. Hemodialysis was carried out for 60 minutes with an ultrafiltration rate of 500 ml/hr. For anticoagulation, heparin was given at a constant infusion rate of 1000 units/hr following an initial bolus dose of 5000 units. No clotting was observed in the extracorporeal circuit in any of the experiments. To avoid hypotension the priming fluid within the extracorporeal circuit (400 ml) was administered to the sheep at the start of dialysis. About four hours after the completion of the experiments the hemodialysis catheters were removed to prevent dislocation or infectious complications. Each sheep was dialyzed with the AN69 membrane (membrane surface area 1.6 m<sup>2</sup>; Filtral<sup>®</sup>, Hospal, Germany), the PAN DX membrane (membrane surface area 1.7 m<sup>2</sup>; PAN-85DX<sup>®</sup>; Asahi Medical, Germany), and the SPAN membrane (membrane surface area 1.8 m<sup>2</sup>; Akzo Nobel Faser AG, Germany) with and without ACE inhibition. The intervals between the experiments were at least three days.

#### Angiotensin converting enzyme inhibition

For ACE inhibition enalapril (Xanef<sup>®</sup>; MSD, Germany) was given daily in two equal doses by oral application starting one week before dialysis. On the day of the experiment enalapril was given 30 minutes before beginning dialysis.

#### Bradykinin B<sub>2</sub>-receptor antagonist

Icatibant (Hoe 140) is known to be a potent and long-acting bradykinin B<sub>2</sub>-receptor antagonist, and efficient in various *in vivo* studies [12]. Recently, it has been shown to prevent anaphylactoid reactions in human low density lipoprotein (LDL) apheresis under ACE inhibition when given prior to the treatment [13]. In the present study icatibant was administered in one defined group of sheep by slow intravenous infusion of 0.1 mg/kg body wt five minutes before dialysis.

#### Test protocol and analytical methods

Eight experimental groups differing with regard to dialyzer type, enalapril dose, and bradykinin B<sub>2</sub>-receptor antagonist were defined. In each group all six animals were tested as follows:

- (1) group AN/0 (*N* = 6), AN69 without ACE inhibition;
- (2) group AN/10 (*N* = 6), AN69 with 10 mg/day enalapril;
- (3) group AN/20 (*N* = 6), AN69 with 20 mg/day enalapril;
- (4) group AN/30 (*N* = 6), AN69 with 30 mg/day enalapril;
- (5) group AN/BA (*N* = 6), AN69 with 30 mg/day enalapril and 0.1 mg/kg bradykinin B<sub>2</sub>-receptor antagonist icatibant (Hoe 140);
- (6) group PA/0 (*N* = 6), PAN DX without enalapril;
- (7) group PA/20 (*N* = 6), PAN DX with 20 mg/day enalapril;
- (8) group SP/20 (*N* = 6), SPAN with 20 mg/day enalapril (control group).

To investigate the extent of contact activation, the concentrations of bradykinin, prekallikrein and high molecular weight kininogen were determined.

After dialysis was started, samples for the determination of bradykinin were drawn from blood entering and leaving the dialyzer at 0, 5, 15, and 60 minutes. In addition, for two animals in groups AN/0 and AN/20, blood samples were also taken from a catheter inserted into a carotid artery prior to the experiment. The samples were collected and processed according to the method of

Shimamoto et al [14]. The radioimmunoassay of kinin was performed as described previously [15].

Blood samples for prekallikrein and high molecular weight kininogen determination were taken at 0, 15, and 60 minutes from venous blood. Preparation of the samples and measurement of prekallikrein was performed as described by Boenner et al [16]. Uncleaved high molecular weight kininogen was measured by a method similar to that described by Uchida and Katoni [17].

For the determination of ACE activity, samples were collected immediately before the start of dialysis. Assays were carried out on plasma by measuring angiotensin II generation using a commercially available test (Hycor Biomedical Inc., Irvine, Ca, USA).

To assess a possible role of histamine in the anaphylactoid reactions, plasma histamine was determined before and 15 minutes after the beginning of hemodialysis using an enzyme immunoassay (Immunotech, AMAC Inc., USA).

Before and after the experimental procedures blood samples for the determination of hemoglobin, platelet count, white blood cells, total serum protein, and activated partial thromboplastin time (APTT) were also drawn.

#### Registration of anaphylactoid reactions

To identify hypotensive episodes during the experiments blood pressures and heart rates were recorded at one minute intervals by an automatic device (Critikon "dynamap" 1846 SX, USA) using a "tail cuff" (neonatal cuff No. 4). This non-invasive method for blood pressure monitoring in sheep has been proven in a previous study to deliver reliable data compared to intra-arterial measurements [10]. The ventilation rate was obtained by counting breaths/min and recorded every five minutes. Furthermore, the animals were observed continuously with regard to symptoms of anaphylactoid reaction like circulatory collapse and edema.

#### Statistical analysis

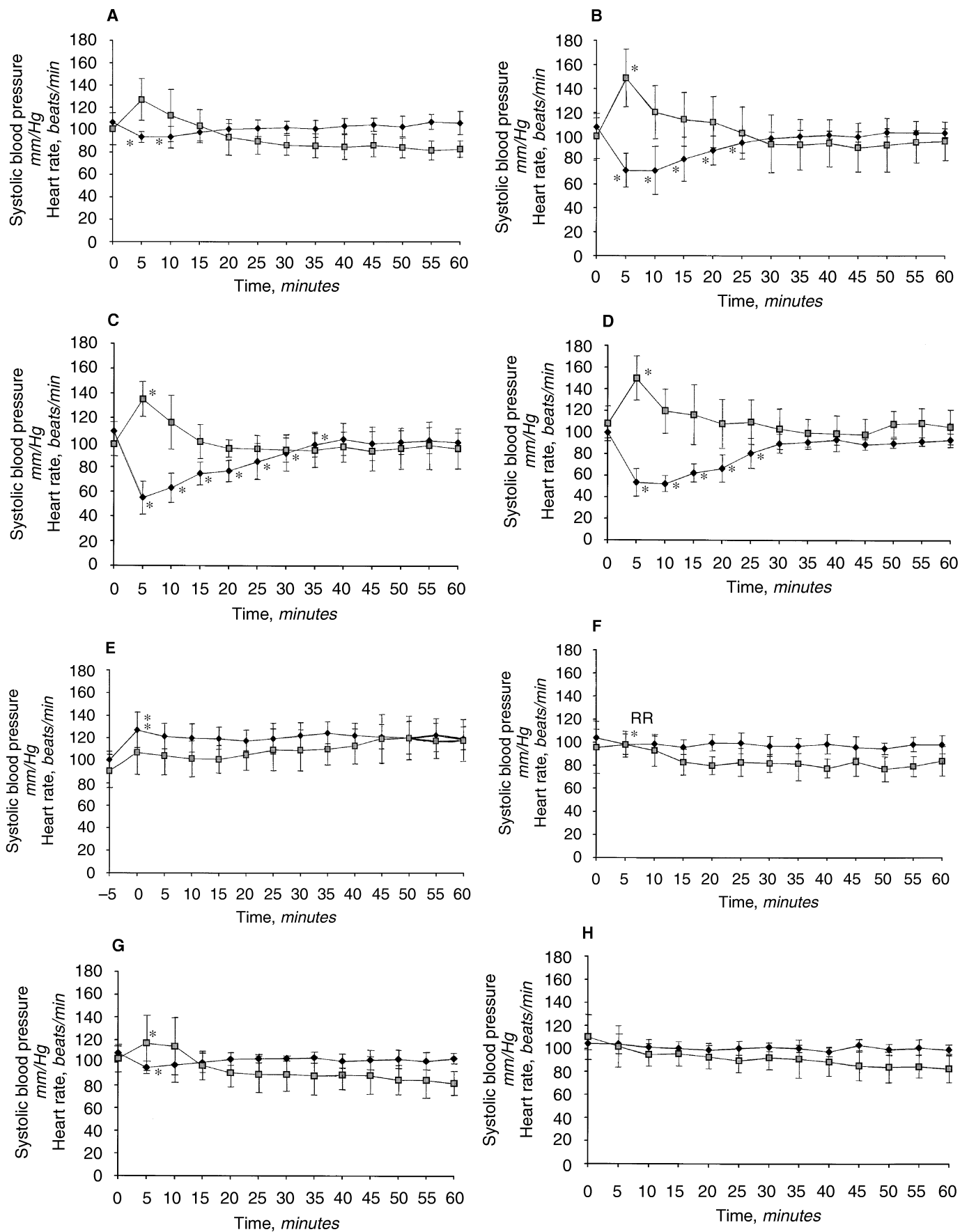
Results are expressed as mean values  $\pm$  SD. Wilcoxon's matched pairs signed rank test was used to compare data within each group. Differences were considered significant at  $P < 0.05$ .

## RESULTS

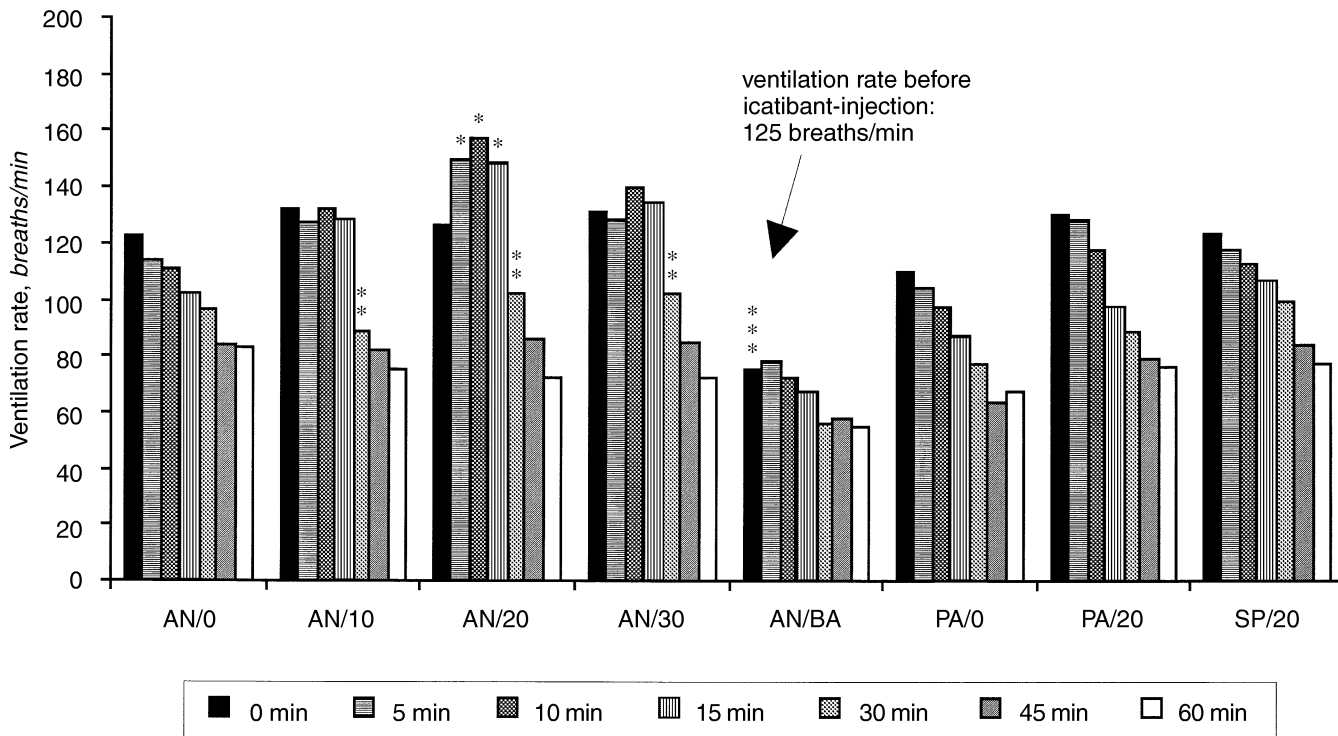
#### Appearance of anaphylactoid reactions

Clinically overt anaphylactoid reactions could be observed only in groups AN/10, AN/20, and AN/30. The symptoms began after five minutes of dialysis and included hypotension leading to restlessness, unsteady standing, and collapsed in the worst case. Furthermore, the sheep showed respiratory problems in the form of prolonged tachypnoea. Edema of the face was not observed. The symptoms were similar in these three groups but differed in frequency and severity. The afflicted animals recovered within 5 to 10 minutes without having obtained any particular treatment like fluid application, etc.

The mildest anaphylactoid reactions could be observed in group AN/10. In this group only one sheep collapsed while three animals showed no symptoms at all. There was a statistically significant fall of systolic blood pressure from  $108 \pm 7.5$  to  $72 \pm 14.2$  mm Hg linked to a significant rise of heart rate from  $100 \pm 18.7$  to  $149 \pm 24.1$  beats/min after five minutes, followed by a continuous recovery of the hemodynamic situation in the further course of dialysis, without changing the treatment mode or application of fluid (Fig. 1B). The ventilation rate in group AN/10 remained



**Fig. 1.** Systolic blood pressure ( $\blacklozenge$ ) and heart rate ( $\blacksquare$ ) during 60 minutes of hemodialysis. Data are mean values  $\pm$  SD of six sheep. (A) Group AN/0; (B) group AN/10; (C) group AN/20; (D) group AN/30; (E) group AN/BA; (F) group PA/0; (G) group PA/20; (H) group SP/20. \* $P < 0.05$  versus 0 minutes, \*\* $P < 0.05$  versus -5 minutes.



**Fig. 2. Ventilation rate of all groups during 60 minutes of hemodialysis.** Data are mean values of six sheep. \* $P < 0.05$  versus 0 minutes; \*\* $P < 0.05$  versus 15 minutes; \*\*\* $P < 0.05$  versus prior to icatibant application.

elevated and almost unchanged during the first 15 minutes of dialysis but fell significantly at 30 minutes (Fig. 2).

In group AN/20 all animals except one showed symptoms: one animal collapsed and the other four had minor signs of anaphylactoid reaction. After five minutes of treatment, a considerable and significant hypotension was observed, with systolic blood pressure falling from  $109 \pm 7.7$  to  $55 \pm 13.4$  mm Hg, and the heart rate increased from  $99 \pm 9.6$  to  $135 \pm 14.1$  beats/min. Until 40 minutes of treatment time the systolic blood pressure was significantly lower compared to the beginning of dialysis (Fig. 1C). The ventilation rate rose significantly with a maximum after 10 minutes ( $127 \pm 17$  vs.  $157 \pm 27$  breaths/min) and normalized gradually after 30 minutes (Fig. 2).

The most severe anaphylactoid reactions could be noted in group AN/30. All six sheep showed severe symptoms, three animals in the form of collapse. There was a significant drop of systolic blood pressure from  $100 \pm 5.6$  mm Hg before dialysis, to  $54 \pm 12.8$  after five minutes reaching a minimum of  $53 \pm 7.5$  mm Hg after 10 minutes of experiment indicating a pronounced state of shock (Fig. 1D). Simultaneously, the heart rate increased significantly from  $108 \pm 16$  to a maximum of  $150 \pm 20.3$  beats/min after five minutes. After 30 minutes of dialysis, blood pressure and heart rate had stabilized without the necessity of interrupting the treatment or application of fluid to the animal. As in group AN/20, the animals of group AN/30 had the maximal ventilation rate after 10 minutes. This increase was not significant compared to the pre-dialysis value ( $132 \pm 11$  vs.  $140 \pm 29$  breaths/min; Fig. 2).

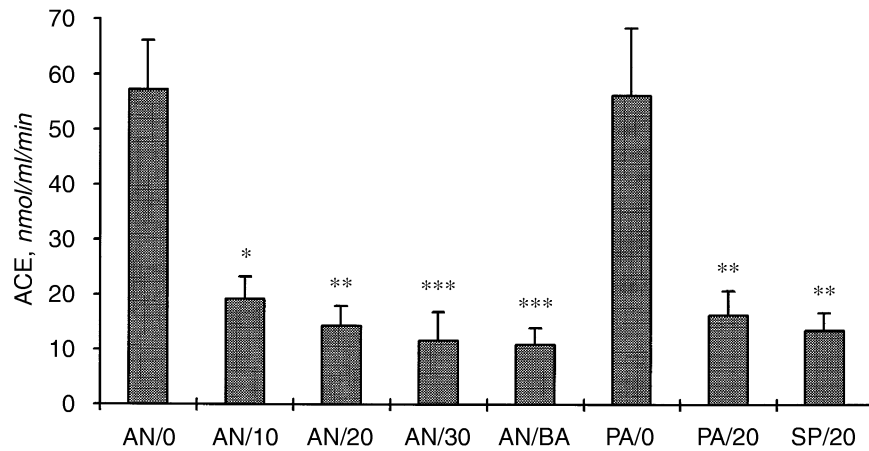
Immediately after application of the bradykinin receptor antagonist (before the start of dialysis) all animals in group AN/BA (AN69 with 30 mg enalapril and icatibant) demonstrated adverse

effects: the symptoms were loss of urine and feces, regurgitation, signs of abdominal discomfort, and hypersalivation that lasted for hours after dialysis. However, it is interesting to note that none of the animals showed symptoms of anaphylactoid reaction during hemodialysis unlike that observed for sheep in groups AN/10 to 30. Furthermore, five minutes after application of icatibant, which was at the beginning of dialysis, a significant rise of systolic blood pressure from  $101 \pm 7.2$  to  $127 \pm 15.9$  mm Hg and an increased heart rate ( $91 \pm 14.9$  to  $107 \pm 19.4$  beats/min) could be observed, but during the whole dialysis time (even in the first 10 min of the experiment) the blood pressure and heart rate remained nearly unchanged (Fig. 1E). Five minutes after the application of icatibant a significant decrease of ventilation rate from  $125 \pm 43$  to  $75 \pm 26$  breaths/min at the beginning of dialysis was observed. During the experiment the ventilation rate fell gradually (Fig. 2).

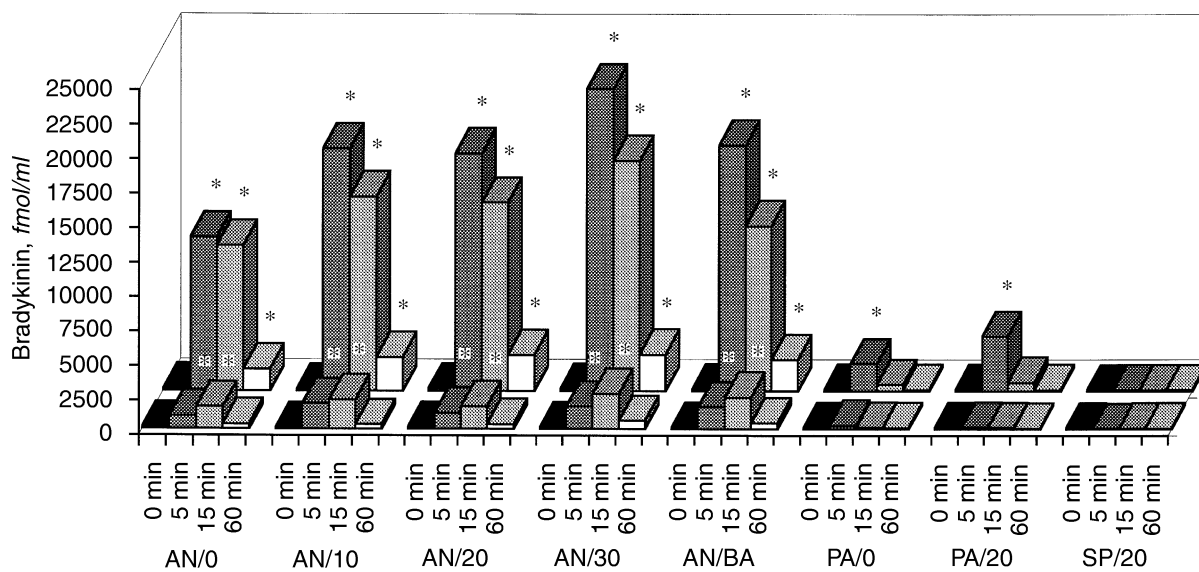
In all other groups (AN/0, PA/0, PA/20, SP/20) any form of symptoms indicative of anaphylactoid reactions were absent.

In groups AN/0 and PA/20 blood pressure and heart rate took an identical course during dialysis. Although there was no real hypotension, a slight but significant decrease of systolic blood pressure from  $107 \pm 8.5$  to  $94 \pm 4.9$  mm Hg in group AN/0 and  $108 \pm 6.3$  to  $96 \pm 5.6$  mm Hg in group PA/20, together with an increase in heart rate ( $101 \pm 14.4$  to  $127 \pm 18.9$  and  $104 \pm 12.2$  to  $117 \pm 24.3$  beats/min, respectively) could be noted after five minutes (Fig. 1A and 1G). The circulation parameters were normalized in both groups after 15 minutes of treatment.

In group PA/0, a minor reduction of systolic blood pressure from  $104 \pm 7.4$  to  $98 \pm 9$  mm Hg established after five minutes. This significant decline in blood pressure was not linked to an increase of the heart rate like in the groups already mentioned



**Fig. 3.** Angiotensin converting enzyme (ACE) activity of all groups before hemodialysis. Data are mean values  $\pm$  SD of six sheep. \* $P$  < 0.05 versus group AN/0 and PA/0 (without ACE inhibition); \*\* $P$  < 0.05 versus group AN/10; \*\*\* $P$  < 0.05 versus group AN/20.



**Fig. 4.** Bradykinin concentrations of all groups before (front columns) and after (rear columns) passage through the dialyzer during 60 minutes of hemodialysis. Data are mean values of six sheep. \* $P$  < 0.05 versus 0 minutes.

(Fig. 1F). Thereafter, hemodynamic parameters remained inconspicuous throughout the course of the experiment.

Blood pressure and heart rate of the animals of group SP/20 did not show any significant changes during the whole treatment period (Fig. 1H).

In groups AN/0, PA/0, PA/20, and SP/20 the ventilation rates were comparable and decreased continuously during the experiment (Fig. 2).

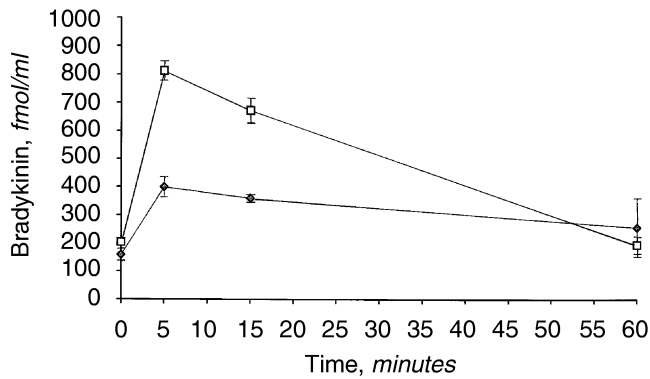
#### Angiotensin converting enzyme inhibition

Figure 3 shows the ACE activity of all groups prior to the experiments. The mean values of the groups without ACE inhibition, groups AN/0 and PA/0, were  $57.26 \pm 8.26$  and  $56.22 \pm 12.29$  nmol/ml/min, respectively. Compared to groups AN/0 and PA/0, a significantly lower ACE activity of  $19.22 \pm 4.06$  nmol/ml/min could be detected in group AN/10. The ACE activity of group AN/20 was  $14.38 \pm 3.57$  nmol/ml/min and differed significantly to group AN/10. The values of group PA/20 and SP/20 ( $16.38 \pm 4.43$  and  $13.6 \pm 3.15$  nmol/ml/min, respectively) were similar to group AN/20 but significantly lower only compared to the groups

without ACE inhibition (AN/0 and PA/0). After 30 mg/day of enalapril, groups AN/30 and AN/BA, the ACE activities amounted to  $11.60 \pm 5.26$  and  $10.99 \pm 2.99$  nmol/ml/min, respectively, and differed significantly to group AN/20.

#### Contact activation

Results of bradykinin determination before and after passage of blood through the dialyzer are given in Figure 4. Most remarkable was an intense and uniform generation of bradykinin after passage of blood through the dialyzer in the groups in which the AN69 membrane was used, reaching its maximum after only five minutes of dialysis. Fifteen and 60 minutes after the beginning of the treatment, the bradykinin concentrations decreased slowly but still remained significantly elevated compared to the pre-dialysis values. The five minutes bradykinin concentrations after passage through the dialyzer were increased 73-fold in group AN/0, 161-fold in group AN/10, 97-fold in group AN/20, 108-fold in group AN/30, and 154-fold in group AN/BA. In these five groups the kinetics of bradykinin levels in the blood samples drawn before passage of the dialyzer were somewhat different: the



**Fig. 5. Bradykinin concentrations in plasma samples obtained from the carotid artery of group AN/0 (AN69 without ACE inhibition;  $\blacklozenge$ ) and group AN/20 (AN69 with 20 mg enalapril;  $\square$ ) during 60 minutes of hemodialysis. Data are mean values  $\pm$  SD.**

highest bradykinin concentrations were measured at 15 minutes after the start of experiment and rose to 10-fold in group AN/0, 14-fold in group AN/10, 9-fold in group AN/20, 17-fold in group AN/30, and 20-fold in group AN/BA compared to the pre-dialysis values. All bradykinin concentrations detected before passage of the dialyzer at 5, 15, and 60 minutes were significantly different from the initial values (0 min). As expected, the highest bradykinin levels, that is, 15 minutes pre-dialyzer and five minutes post-dialyzer, had been measured in group AN/30 and the lowest in group AN/0 whereas the bradykinin concentrations in group AN/10 and group AN/20 were not ACE inhibitor dose-dependent. In group AN/10 the bradykinin concentrations were markedly higher compared to group AN/20. Thus, there was no correlation between the dose of ACE inhibitor and the amount of bradykinin generation before or after passage of blood through the dialyzer. Compared to group AN/0, the bradykinin levels at 5 and 15 minutes of treatment after the dialyzer in groups AN/10, AN/20, AN/30 and AN/BA were significantly different.

To demonstrate the influence of ACE inhibition on arterial bradykinin concentrations during hemodialysis with the AN69 membrane, additional arterial blood samples were drawn in groups AN/0 and AN/20. In both groups the maximum levels were reached at five minutes meaning a 2.5-fold increase in group AN/0 and a fourfold increase in group AN/20 (Fig. 5). Fifteen minutes after beginning of dialysis, the bradykinin concentrations were still markedly elevated and at 60 minutes the values were comparable to 0 minutes (Fig. 5).

Hemodialysis with the PAN DX membrane was also associated with a bradykinin release although not to the same extent as AN69. In group PA/0 and PA/20 a significant 18-fold and 42-fold increase, respectively, of bradykinin concentrations after passage through the dialyzer could be observed five minutes after the start of dialysis (Fig. 4). The difference in bradykinin generation between groups PA/0 and PA/20 at five minutes was significant. A significant elevation of bradykinin levels after passage of the dialyzer was also detectable at 15 minutes in group PA/20. Before passing the dialyzer bradykinin levels were not clearly different at any time in both groups (Fig. 4).

The SPAN membrane did not lead to significant changes in bradykinin concentrations, either before or after, passage of blood through the dialyzer (Fig. 4).

To show the extent of contact activation, the plasma levels of prekallikrein and HMW kininogen were determined.

A significant decrease of prekallikrein concentrations could be observed in all groups except group SP/20 where no significant changes could be demonstrated. Fifteen minutes after the beginning of dialysis, this reduction in prekallikrein concentration was 13.4% in group AN/0, 13.8% in group AN/10, 8% in group AN/20, 29.1% in group AN/30, and 30.6% in group PA/0 (Fig. 6). In these groups, the prekallikrein levels at 60 minutes were similar to the 15 minute levels, and were significantly lower compared to the pre-dialysis values (Fig. 6).

The fall of high molecular weight kininogen levels was even more pronounced and detectable in groups AN/0 to 30 and PA/0. In group SP/20 the high molecular weight kininogens levels did not differ significantly in the course of dialysis (Fig. 7). After 15 minutes of treatment the significant reduction of high molecular weight kininogen was 47% in group AN/0, 42% in group AN/10, 46% in group AN/20, 42% in group AN/30, and 25% in group PA/0. At 60 minutes high molecular weight kininogen concentrations were only slightly decreased in these groups (Fig. 7).

#### Further parameters

The evaluation of plasma histamine levels, total protein, and white blood cells yielded no changes during the experiments (data not shown).

The mean values of hemoglobin concentrations  $\pm$  SD before dialysis were between  $9.7 \pm 1.0$  and  $10.9 \pm 1.4$  g/dl. At the end of dialysis the concentrations were diminished in all groups to values between  $9.3 \pm 0.8$  and  $9.6 \pm 0.6$  g/dl (not significant).

The platelet count took a similar course. Before dialysis platelet counts between  $320 \pm 108$  and  $393 \pm 87 \times 10^3/\mu\text{l}$  were measured, while at 60 minutes the mean values  $\pm$  SD were reduced to  $270 \pm 103$  and  $318 \pm 135 \times 10^3/\mu\text{l}$ . These differences were not significant in all groups.

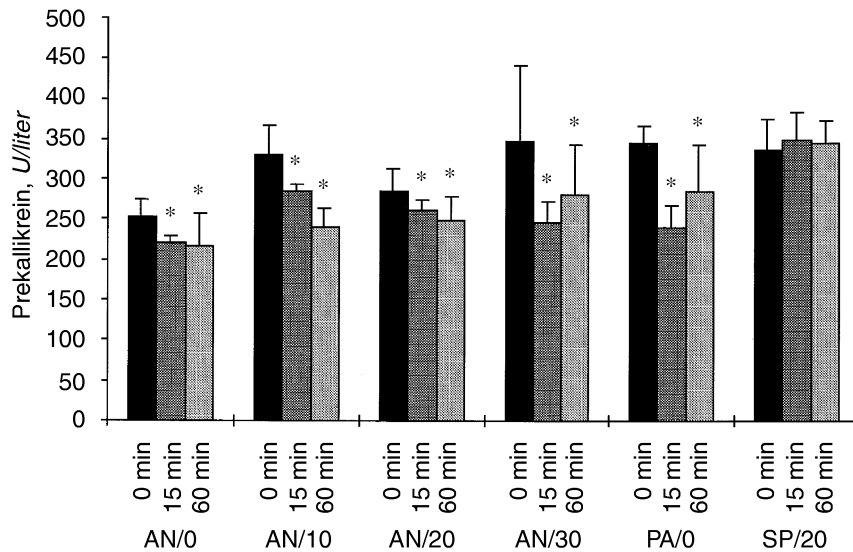
The mean values  $\pm$  SD of activated thromboplastin time (APTT) of the groups before experiment were between  $25 \pm 5$  and  $45 \pm 34$  seconds. After an initial dose of 5000 IU heparin and a continuous rate of 1000 IU/hr, the increment of APTT at 60 minutes was significant in all groups ( $176 \pm 155$  to  $370 \pm 170$  seconds). No significant clotting problems occurred.

## DISCUSSION

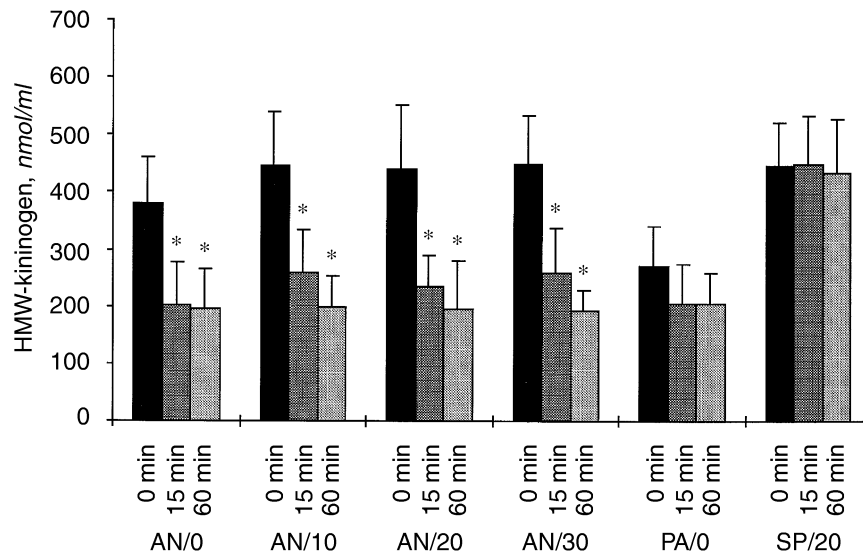
### The bradykinin hypothesis

Recent studies have demonstrated the possible key role of bradykinin in anaphylactoid reactions during hemodialysis with the AN69 membrane in patients on ACE inhibitors [8–11]. However, definitive evidence that bradykinin is the mediator of the observed anaphylactoid reactions has been lacking, causing much controversy. Such evidence would be convincing when an inhibitor of the postulated mediator could abolish the symptoms of anaphylactoid reaction.

AN69 membranes are capable of generating bradykinin when incubated with human blood [8, 9, 18]. This property may be related to the negative charge of the membrane surface that causes generation of active plasma kallikrein via contact activation [9, 19, 20]. Plasma kallikrein, in turn, cleaves high molecular weight kininogen under release of bradykinin. In the presence of the inhibitors of ACE (= kininase II), the major enzyme of kinin



**Fig. 6. Prekallikrein concentrations of groups AN/0, AN/10, AN/20, AN/30, PA/0, and SP/20 during 60 minutes of hemodialysis. Data are mean values  $\pm$  SD of six sheep. Symbols are: (■) 0 min; (▨) 15 min; (▩) 60 min. \* $P < 0.05$  versus 0 minutes.**



**Fig. 7. High molecular weight kininogen concentrations of groups AN/0, AN/10, AN/20, AN/30, PA/0, and SP/20 during 60 minutes of hemodialysis. Symbols are: (■) 0 min; (▨) 15 min; (▩) 60 min. Data are mean values  $\pm$  SD of six sheep. \* $P < 0.05$  versus 0 minutes.**

inactivation *in vivo* [21], bradykinin, passes the pulmonary circulation without being inactivated and accumulates to high concentrations that are able to evoke anaphylactoid reactions [8]. This “bradykinin hypothesis” was supported by several *in vivo* studies. Recently, we have shown in an animal model that hemodialysis with the AN69 membrane is associated with a bradykinin release in blood after passage through the dialyzer [10]. Pre-treatment with the ACE inhibitor captopril led to further increases in bradykinin levels and resulted in anaphylactoid reactions [10]. Verresen et al demonstrated elevated bradykinin concentrations in blood from the arterial line of the extracorporeal circuit in nine patients under ACE inhibition suffering from anaphylactoid reactions during hemodialysis with AN69 dialyzers [11]. Although the “bradykinin hypothesis” is backed by some experiments by several groups, a number of issues remain unclear. Firstly, there are a number of case reports about patients not experiencing anaphylactoid reactions despite usage of AN69 dialyzers together with pre-treatment with ACE inhibitors [22–26]. Such observa-

tions have led to the discussion about the varying individual susceptibility for anaphylactoid reactions and a possible incomplete inhibition of kininase II due to a low dosage of ACE inhibitors [9]. On the other hand, there is information about at least four cases of severe anaphylactoid reactions and dialysis with AN69 dialyzers without previous intake of ACE inhibitors [11, 25, 27]. In two of these patients excessive concentrations of bradykinin were detected in the venous effluent of the dialyzers during these episodes, strongly suggesting bradykinin as the mediator of the observed reactions [11, 27]. The reason for the inability of these patients to degrade bradykinin without present ACE inhibitor medication is not known.

#### Bradykinin as the mediator of anaphylactoid reactions

The results of our animal study further support the key role of bradykinin in the pathogenesis of anaphylactoid reactions during hemodialysis with the AN69 membrane and pre-treatment with ACE inhibitors. Confirming other clinical, animal, and *in vitro*

studies [9–11, 28], we show that hemodialysis with AN69 is associated with an excessive generation of bradykinin with a peak after five minutes of treatment in the venous effluent of the dialyzer, with or without ACE inhibitor.

Elevated bradykinin levels were also found at the dialyzer inlet. Probably due to systemic accumulation but possibly because coating of the membrane by proteins may already have reduced the ability of the AN69 membrane to generate bradykinin, maximum concentrations were detected only after a delay of 15 minutes. Increased bradykinin concentrations before passage of the dialyzer were also shown by Verresen et al in patients with anaphylactoid reactions with and without intake of ACE inhibitors [11]. In contrast, another study performed with patients under pre-treatment with ACE inhibitors and hemodialysis with AN69 dialyzers demonstrated normal bradykinin concentrations in the arterial line and only slightly increased concentrations in the venous line of the extracorporeal circuit. Interestingly, none of these patients had any anaphylactoid reactions [28]. Taken together, it could be speculated that bradykinin detection in samples drawn from the arterial line of the extracorporeal circuit represents approximately systemic arterial concentrations and may be extrapolated to the observed anaphylactoid reactions.

Since kinins would be inactivated during first passage of the pulmonary circulation, high bradykinin concentrations in blood after passage through the dialyzer do not have any predictable value on arterial bradykinin levels [29]. Furthermore, it is known that anaphylactoid reactions in the form of a significant decrease in systemic blood pressure occur when physiological kinin concentrations increase about five- to tenfold in the arterial branch of the circulation [30, 31]. Therefore, we investigated bradykinin levels in blood samples drawn directly via a small catheter from the carotid artery. The puncture and insertion of this catheter in non-anesthetized sheep was extremely difficult and exhausting for the animals, so we had to limit this procedure to only two sheep of group AN/0 and group AN/20 in which anaphylactoid reactions had been expected. Arterial bradykinin concentrations in group AN/0 increased five minutes after initiation of hemodialysis 2.5-fold, and were accompanied by a slight hypotension and tachycardia, suggesting a minor anaphylactoid reaction due to excessive bradykinin generation exceeding the inactivation capacity of the pulmonary circuit despite absent ACE inhibition. Arterial bradykinin concentrations of group AN/20 were fourfold increased at five minutes, at a time when severe anaphylactoid reactions in the form of collapse occurred. This observation strongly supports that bradykinin is the mediator of the anaphylactoid reactions.

#### Effect of the bradykinin B<sub>2</sub>-receptor antagonist icatibant

Icatibant (Hoe 140) is known as a potent and long-acting bradykinin antagonist. It prevents bradykinin-induced hypotension and bronchoconstriction in different *in vivo* models and in humans [12, 32, 33]. Furthermore, icatibant has been successfully applied as an antidote for anaphylactoid reactions during dextran sulfate LDL apheresis in a patient under ACE inhibition [13]. This led us to use icatibant in one group of animals pretreated with 30 mg/day enalapril and hemodialyzed with AN69 (group AN/BA) to prove bradykinin as the mediator of the anaphylactoid reactions. After application of icatibant before hemodialysis, we could observe an increase of blood pressure and heart rate and a marked reduction of tachypnoea. During hemodialysis neither hypotension, tachycardia, nor collapse occurred, and the ventila-

tion rate fell continuously despite high pre- and post-dialyzer bradykinin levels that were comparable to those of group AN/30, in which the most severe anaphylactoid reactions had been noted. These results provide evidence that bradykinin is the mediator of anaphylactoid reactions during hemodialysis with the AN69 membrane.

However, the application of icatibant was not without adverse effects that persisted for hours even after termination of dialysis due to the extended duration of icatibant action. In every case these adverse effects appeared before the start of dialysis and corresponded to symptoms of an intestinal action of bradykinin. These symptoms were similar to adverse effects of Icatibant observed during a tolerability testing in dogs and were attributed to a residual bradykinin B<sub>2</sub>-receptor agonistic activity [12, 34]. Since the exact dose of icatibant necessary to prevent bradykinin-induced reactions in sheep was not known, based on recent *in vivo* studies we decided to use 0.1 mg/kg. Most probably, lower icatibant doses would have been better tolerated but the observed adverse effects were of minor importance for our examinations, so we were not motivated to alter the dose during the study.

The increase of blood pressure and heart rate after application of icatibant before dialysis had been most probably a reaction of the animals due to the observed intestinal symptoms. On the other hand, a contracting effect of icatibant on the endothelium-free femoral artery of sheep is known [35]. Therefore, a partial agonistic effect of icatibant on arterial vessels cannot be excluded and may have been involved in the pre-dialytic blood pressure increase and prevention of hypotension after the start of dialysis. In group AN/30, the hypotension after the beginning of dialysis was extreme. Since we could not observe even a slight decrease of blood pressure during dialysis in group AN/BA, the absence of hypotension can only be due to the antagonistic effect of icatibant on the endothelium-derived vasodepressive action of bradykinin.

#### Angiotensin converting enzyme inhibitor dose and anaphylactoid reactions

To verify the effect of different kininase II inhibition (ACE activity) on anaphylactoid reactions and contact activation in our study, hemodialysis with AN69 was performed at different doses of enalapril. Compared to humans, ACE activity in the group of sheep without enalapril (group AN/0) was in the normal range [36]. Administration of enalapril led to a significant dose-dependent reduction of ACE activity. Although daily administration of 10 mg enalapril resulted in an already therapeutic reduction of ACE activity, increasing the doses (20 and 30 mg/day enalapril) intensified ACE inhibition.

There seems to be a direct dependency between the enalapril dose and ACE activity and the severity of anaphylactoid reactions in hemodialysis with the AN69 membrane. While hemodialysis without ACE inhibition was nearly uneventful, clinically overt anaphylactoid reactions could be observed even with as low as 10 mg enalapril. With increasing enalapril doses anaphylactoid reactions became more frequent and severe; with 30 mg enalapril (group AN/30) all sheep suffered from anaphylactoid reactions. Probably due to a rapid down-regulation of the bradykinin B<sub>2</sub>-receptor through an excess of bradykinin and the potent compensatory action of the sympathetic nervous system, all sheep recovered after a few minutes without the need of intervention and despite continuation of the experiment [37].

A comparison of the enalapril-treated groups did not show a direct correlation between the dose of enalapril and levels of



bradykinin. This discrepancy may be explained by the lack of linearity between the dose of enalapril and ACE inhibition. A dose of 10 mg enalapril results in ACE inhibition in the therapeutic range, while a further increase of the enalapril dose leads to only a slight additional reduction of ACE activity. This produces an inadequate increase of bradykinin generation, which is massive even without ACE inhibition. Furthermore, systemic bradykinin accumulation may not be the only reason for the severity of anaphylactoid reactions, since bradykinin levels after 10 mg of enalapril are probably sufficient to provoke more severe anaphylactoid reactions than observed. Due to ACE inhibition, the potent counter-regulatory action of angiotensin II is diminished [38]. This effect adds to the severity of the anaphylactoid reactions and may explain their dose-dependency on enalapril.

Furthermore, it has been shown that ACE inhibition increases circulating plasma levels of vasodilators other than bradykinin, such as prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and substance P, which could aggravate hypotension during anaphylactoid reactions [39].

#### PAN-membrane type and anaphylactoid reactions

*In vitro* experiments by Lemke and Fink revealed that in addition to the AN69 membrane the PAN DX membrane also generates bradykinin in remarkably high amounts [8]. In our study we could confirm the bradykinin generating property of the PAN DX membrane *in vivo*. The kinetics of bradykinin concentrations during hemodialysis with the PAN DX membrane were comparable to AN69, but the extent of bradykinin levels was less pronounced, resulting in only minor anaphylactoid reactions after pretreatment with 20 mg enalapril. This contact activation of PAN DX could be attributed also to a negatively charged membrane surface and, since this negative charge may be reduced compared to AN69, the release of bradykinin is reduced [8]. In fact, negative surface charge was shown to increase in the sequence SPAN < PAN DX < AN69 when these membranes were compared in a measurement of the zeta-potential between pH 3 to 10 (Werner C, Tulke A, Lemke HD; manuscript in preparation).

Our study shows that hemodialysis with the AN69 and PAN DX membranes causes significant contact activation, as indicated by the generation of bradykinin and the consumption of the contact system components prekallikrein and high molecular weight kininogen during the first 15 minutes of dialysis. Between 15 and 60 minutes of dialysis prekallikrein and high molecular weight kininogen levels remained nearly unchanged, indicating a reduction of contact activation due to coating of the membrane surface. While the concentrations of prekallikrein and high molecular weight kininogen during hemodialysis with AN69 and PAN DX were nearly similar and bradykinin levels were much higher during treatment with AN69, it is tempting to speculate that, in contrast to AN69, the PAN DX membrane is capable of adsorbing high amounts of bradykinin.

Several *in vitro* and *in vivo* studies have shown that the uncharged polyacrylonitrile membrane SPAN does not generate bradykinin [10, 40, 41]. Furthermore, hemodialysis of ACE inhibited patients and sheep with this membrane did not provoke anaphylactoid reactions [10, 41]. Therefore, in our experimental setting SPAN was used as a control membrane only in enalapril pretreated animals. All experiments with the SPAN membrane were clinically uneventful and without changes of bradykinin concentration. Since there was no alteration of prekallikrein and high molecular weight kininogen levels, a contact activation

combined with a possible adsorption of bradykinin by SPAN can be excluded.

#### Other possible mechanisms of anaphylactoid reactions

Recently, an influence of the calcium composition of the dialysate on anaphylactoid reactions was suggested. Low dialysate calcium concentrations (1.25 mmol/liter) were associated with anaphylactoid reactions in patients on ACE inhibitor therapy and hemodialysis with the AN69 membrane, whereas changing the calcium concentration to 1.75 mmol/liter resulted in symptom-free dialysis in previously symptomatic patients implicating a possible, direct inhibitory effect of calcium on bradykinin activity [24]. To exclude the influence of low dialysate calcium concentrations in our experimental setting, a dialysate calcium concentration of 1.75 mmol/liter, able to prevent anaphylactoid reactions in human hemodialysis patients, was chosen.

Furthermore, we could exclude a suggested effect of histamine release on the observed anaphylactoid reactions since histamine levels remained unaltered during all experiments [25], thereby confirming the results of *in vitro* studies and of our previous animal study [9, 10].

To avoid early hypovolemic effects that could interfere with bradykinin-mediated anaphylactoid reactions, the priming fluid of the extracorporeal circuit was administered to the sheep with the beginning of dialysis. In fact, this procedure prevented blood pressure alterations as seen in the control group (group SP/20) in which no bradykinin was released. At the onset of anaphylactoid reactions in all other groups only about 50 ml of fluid were withdrawn, a volume unlikely to evoke hypotension. However, apart from the amount of blood samples taken during the experiments, the infusion of the priming fluid could have contributed to the slight reduction of the hemoglobin concentrations and the platelet counts at the end of dialysis.

#### Conclusion

Our animal model of hemodialysis with sheep proved to be suitable for the testing of contact activation. Compared to human data this species seems to be extraordinarily sensitive to contact activation with a vigorous release of bradykinin [10, 11, 27, 28]. The results of this study confirm *in vitro* and *in vivo* studies that anaphylactoid reactions during hemodialysis with AN69 membranes are mediated by bradykinin, since they can be prevented by the bradykinin B<sub>2</sub>-receptor antagonist icatibant (Hoe 140). The severity of these anaphylactoid reactions does not depend on the maximum of bradykinin levels generated by the membrane, but on the dose of ACE inhibitor and the subsequent ACE activity. The PAN DX membrane is also capable of activating the contact system and to release bradykinin although to a lower extent. Hemodialysis with the SPAN membrane is not associated with any contact activation.

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