Preventive strategies in endothelin-induced renal failure

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Preventive strategies in endothelin-induced renal failure. The endothelial vasoconstrictor endothelin (ET) can induce acute renal failure when fibrinolysis and vasodilatory prostanoids (PGs) are inhibited. This study compares therapeutic agents preventing ET-induced acute renal failure in anesthetized female pigs. We investigated the effect of four ET boli (1.5 μg/kg, i.v.) after pretreatment with indomethacin (2 mg/kg) and ϵ-aminocaproicacid (100 + 50 mg/kg) alone (controls, group 1) or during additional nifedipine (10 μg/kg/h; group 2), hirudin (0.5 mg/kg; group 3), or enalapril (2 × 0.15 mg; group 4) on coagulation, PGs, and renal function. The ET-induced blood pressure increase was lower in groups 2 to 4 (lowest in group 3, $P < 0.05$). PG synthesis was blocked in all groups. The initial hypercoagulability (controls) resulted in disseminated intravascular coagulation that was prevented by hirudin and was attenuated in groups 2 and 4. At the end of the experiment, creatinine clearance was significantly ($P < 0.05$) decreased. The recovery of renal function two hours after the last ET bolus was most pronounced in the hirudin group. All therapeutic drugs attenuated ET-induced impairment of renal function. Hirudin seems to be the most potent protective drug. Prevention of further ET release evoked by ET-mediated secretion of thrombin might explain this. These results suggest three important pathways for ET's hemodynamic and renal effects.

Beside its vasoactive effects, endothelin (ET) interacts, especially in the kidney, with the prostaglandin (PG) system and the endogenous coagulation system. Previous studies have shown that when protective fibrinolysis and PG synthesis are inhibited, exogenous administration of ET to plasma levels two to three times higher than in healthy subjects can induce renal failure [1]. Such high levels occur in disease states such as hepatorenal syndrome and septic shock. In this model, we compared the effect of a calcium channel blocker [nifedipine (NIF)], a direct thrombin antagonist [hirudin (HIR)], and an angiotensin-converting enzyme inhibitor [enalapril (ENAL)] on ET-induced changes in renal function, blood coagulation, and hemodynamics. HIR was used because of the well-documented activation of ET release by thrombin [2, 3].

METHODS

Female, domestic, Göttingen-bred pigs (33.4 ± 2.5 kg, $N = 20$) were premedicated with ketamine (20 mg/kg, i.m.) and atropine (0.02 mg/kg, i.m.), anesthetized with piritramide (10 mg i.v. plus 0.1 mg/kg/hr i.v.), and ventilated with O_2, N_2O, and halothane using a positive-pressure respirator. The right internal carotid artery was connected to a transducer system for continuous analysis of blood pressure (BP) and heart rate. After three 20-minute blood samples for baseline measurements, the experiment was started. Drugs were given i.v. at fixed times in four groups. All groups received ET (1.5 μg/kg; 0, 60, 120, and 180 minutes), indomethacin (2 mg/kg, to inhibit PG synthesis, −10 min), ϵ-aminocaproicacid (100 + 50 mg/kg to inhibit fibrinolysis, −15 and 105 minutes). Group 1 (control) received no other drugs. Group 2 received NIF (10 μg/kg/h, −10 min), and group received 3 ENAL (2 × 0.15 mg, −10 and 110 min) and group 4 HIR (0.5 mg/kg, −10 and 110 min). Blood gas analysis, electrolyte, and creatinine clearance measurements were performed hourly. At 10, 30, and 60 minutes after each ET bolus, blood samples were taken for determination of plasma-renin activity, 6-keto-PGF$_{1\alpha}$, PGE$_2$, thromboxane-B$_2$, ET-1, and coagulation parameters. ET and plasma renin activity were measured by radioimmunoassay, plasma PGs by enzyme immunoassay, coagulation parameters as described elsewhere [4], and proteinuria by a nephelometer analyzer (Behringwerke, Marburg, Germany). Statistical analysis was carried out using commercial software (SAS, Cary, NC, USA). The normal distribution of variables was tested by the Shapiro-Wilk test. If the variable was normally distributed, the t-test for paired samples was used. If not, the Wilcoxon matched-pairs rank test was applied. Significance was set at $P < 0.01$ for blood gas analysis and proteinuria and $P < 0.05$ for other parameters.

RESULTS AND DISCUSSION

In all groups, the second ET bolus evoked the largest BP increase: in controls increase was from (systolic/diastolic) 88.6/61.6 ± 13.1/15.9 to 189.6/142.3 ± 4.5/13.5 mm Hg. HIR had the strongest antihypertensive effect. (BP after the second ET bolus was 139.2/107 ± 15.6/12.8 mm Hg.)
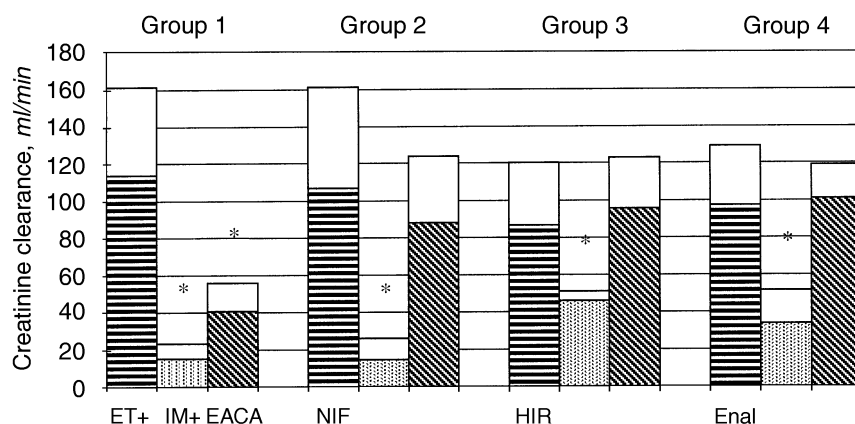
Fig. 1. Creatinine clearance in the experimental groups. After a 60-minute equilibration period, all groups received indomethacin (IM; 2 mg/kg, −10 minutes) and ε-aminocaproic acid (100 + 50 mg/kg, −15 and 105 minutes). Group 1 (left) received no other pretreatment. Group 2 (left center) received additional nifedipine (NIF; 10 μg/kg/h, starting at −10 minutes), whereas group 3 (right center) received hirudin (HIR; 0.5 mg/kg, −10 and 110 minutes), and group 4 (right) received enalapril (ENAL; 2 × 0.15 mg/kg, −10 and 110 minutes). All groups were given four boli (0, 60, 120, and 180 minutes) of endothelin (ET; 1.5 μg/kg). For each group, the three bars represent the times: 0, 240, and 300 minutes. The shaded area represents the value. The open area represents the SD. *$P < 0.05$ versus 0 minutes.

NIF and ENAL reduced the maximum increase in BP by about 15 mm Hg. ET elicited a continuous decrease in blood pH and standard bicarbonate during the experiment, with different degrees of recovery in the postdrug period. At 240 minutes, significant proteinuria was present in all groups, with good recovery in groups 2 to 4 (total recovery in group 4) and an ongoing increase up to 912.6 ± 275.3 mg/liter in controls. At the end of the experiment, creatinine clearance was decreased in all groups with maximum decrease in controls followed by groups 2 and 4. Creatinine clearance in groups 2, 3, and 4 recovered to baseline in the postdrug period, compared with 40.5 ± 15.4 ml/min in group 1 (Fig. 1). Plasma ET was significantly lower in the treatment groups than in control, especially in the NIF and the HIR groups. ET activated the endogenous coagulation system, resulting in disseminated intravascular coagulation in controls [lengthening of the activated partial thromboplastin time, a decrease in antithrombin III (AT III) and factor VIII activity after a transient slight increase after the first three ET boli]. These effects were attenuated in groups 2 and 4 and abolished in group 3 in which activated partial thromboplastin time was lengthened initially to 42.4 ± 3.32 seconds and subsequently returned to baseline at the end of the experiment.

To summarize the findings, all therapeutic drugs had a hypotensive effect. NIF blocks the voltage-operated dihydropyridine-sensitive Ca^+ channels activated by ET [5]. Other studies have shown that NIF inhibits ET-stimulated renin release [6]. ENAL inhibits basal and stimulated secretion of ET [7], possibly through an increase of endogenous nitric oxide (NO) via bradykinin. A further possibility is inhibition of the renin-angiotensin system by inhibition of angiotensin-converting enzyme and weakening of ET-induced potentiation of the vasoconstrictive effects of Ang II. ENAL achieved the greatest protection against ET-induced metabolic acidosis and proteinuria, which emphasizes the importance of an intact renal endothelium and adequate glomerular blood flow. Through its preferential vasodilatory effect on the efferent arterioles mainly mediated by NO and bradykinin, ENAL protects the glomerulus against the massive ET-induced BP increase [8, 9]. The remarkable effects of HIR are still not completely understood. Thrombin inhibits the neutral endopeptidase that is responsible for the degradation of peptide hormones such as ET [2]. HIR, as a direct inhibitor of thrombin, might abolish these effects and accelerate degradation of ET. In view of the ET-induced increase in thrombin-antithrombin complexes and decrease in AT III activity [10], the lower plasma ET-levels and the attenuated decrease in AT III levels in the HIR group also support these considerations. These data show clearly the interaction of coagulation and endothelium-derived vasoactive factors such as ET. A diffuse microthrombosis induced by the activation of coagulation also may play a role in the reduction of renal function. This effect, as well as the ET-induced BP increase, might be affected by HIR therapy, preventing further ET release evoked by ET-mediated secretion of thrombin.

In conclusion, HIR seems to be the most potent protective drug. The prevention of further ET release evoked by ET-mediated secretion of thrombin could serve as an explanation. The efficacy of all therapeutic drugs suggests three important pathways of ET's hemodynamic and renal effects.

ACKNOWLEDGMENTS

APPENDIX

Abbreviations used in this article are: AT III, antithrombin III; BP, blood pressure; ENAL, enalapril; ET, endothelin; HIR, hirudin; PG, prostaglandin; NIF, nifedipine; NO, nitric oxide.

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