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The endothelium-derived relaxing factor-mediated acetylcholine response of the arterial perfusion pressure after cold storage of the isolated rabbit kidney

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Abstract The vasodilatation induced by acetylcholine (ACh) in a rabbit isolated perfused kidney was abolished when the tissue was exposed to cold ischemia for 72 h in Euro-Collins (EC) solution. This vasodilatation is due to the release of endothelium-derived relaxing factor (EDRF) from renal vasculature as evidenced by the attenuation following methylene blue pretreatment. When kidneys were preserved in EC solution containing UK 38485, a thromboxane synthase inhibitor, or nicardipine, a calcium channel blocker, ACh-induced vasodilatation persisted after 72 h of cold ischemia. These results were taken as evidence of tissue protective activity of UK 38485 and nicardipine and have promising implications for cadaveric kidney transplantation.

Key words Kidney preservation, endothelium-derived relaxing factor · Endothelium-derived relaxing factor, kidney preservation · Preservation, kidney, endothelium-derived relaxing factor

Introduction

Damage to glomeruli, tubules, and kidney vascular endothelium has been described following hypothermic storage of kidneys [11, 13] and this, in turn, adversely affects the outcome of transplantation [3, 6, 10]. It is now generally accepted that the endothelium plays a significant role in controlling the underlying vascular smooth muscle and that it acts as an endocrine organ [4]. Endothelium-derived relaxing factor (EDRF), which has been identified as nitric oxide (NO) [15], is a potent vasodilatator and anti-aggregating substance [2], and it may have a crucial role in several cardiovascular diseases [20]. The vasodilator action of EDRF (NO) is mediated through the activation of soluble guanylate cyclase, causing the production of c-GMP in vascular smooth muscle [12, 16]. Methylene blue (MB), a potent inhibitor of soluble guanylate cyclase [14], abolishes this vasodilator effect of acetylcholine (ACh) [9].

The exact mechanisms involved in both ischemia-reperfusion injury and graft rejection are not clear, but there is some evidence that eicosanoids, especially thromboxane A_2 [TxA_2], and increased intracellular calcium may play a role in this damage. Moreover, the cytoprotective roles of thromboxane synthetase inhibitors and calcium channel blockers on reperfusion injury and allograft rejection have previously been reported [1, 5, 7, 18, 19].

The aims of the present study were to assess the vasodilator effect of ACh, which is mediated by EDRF [NO], on the kidney vascular endothelium exposed to cold ischemia and to assess the roles of UK 38485; a thromboxane synthetase inhibitor, and nicardipine, a calcium channel blocker, on this secretion.

Materials and methods

The protocol of the present study was evaluated and approved by the Animal Ethics Committee of the Medical Faculty of Ankara University. The experiments were performed on isolated perfused kidneys from adult rabbits of both sexes weighing 1.5–2.5 kg. The animals were anesthetized with sodium pentobarbital (30 mg/kg i.v.). Both kidneys were removed immediately after the anesthesia was established. Then, the renal artery was cannulated with polyethylene tubing. This procedure usually took 1–2 min, which was considered to be the warm ischemia period for the kidney. After that, the kidneys were flushed out via the cannulated artery with the following hypothermic (2–4 °C) solutions: standard Euro-Collins (EC) solution ($n = 7$, control group); EC containing UK 38485 (10^{-6} M), a thromboxane synthetase inhibitor ($n = 5$); and EC solution containing nicardipine (10^{-7} M), a calcium channel blocker ($n = 6$). One of the kidneys from each rabbit was placed in the preservation solution while the other was kept as a control. Then the kidneys were transferred to a beaker containing the same preservation solutions and kept for 72 h under hypoxic, hypothermic conditions (2–4 °C). At the end of the hypothermic storage, the kidneys were perfused with warmed (37 °C), gassed (5 % CO_2 in O_2), freshly prepared Krebs solution, as previously described from the same research laboratory [19]. Perfusion was provided via a Harvard peristaltic pump, delivering 10–15 ml/min constant flow throughout the experiments. Perfusion pressure (PP) was measured with a pressure transducer (Statham P23 Dc) and recorded on a Grass polygraph (Model 79D). The UK 38485 used in this study was provided by Pfizer (Kent, UK), the nicardipine by Sandoz (Basel, Switzerland), and the phenyleprine hydrochloride (Phe) and acetylcholine chloride (ACh) by Sigma (USA).

All kidneys were first perfused with Krebs solution for an equilibration period of about 60 min. During this period, stable baselines in PP (60–80 mmHg) were established. Submaximal vasoconstriction (about 75 % of maximum) was elicited by phenyleprine (Phe, 10^{-7} M) when added to the perfusion medium. After the establishment of a plateau response in PP, bolus injections of ACh were made into the circuit just proximal to the cannulated renal artery in order to investigate the diminution of the PP, which was mediated by EDRF (NO). The experiments were repeated on kidneys pretreated with MB added to the perfusion medium at a concentration of 10^{-5} M for 20 min in order to inhibit soluble guanylate cyclase activity [14].

The changes in PP were measured on the recorder and expressed as Δ mmHG. Values are given in the text and figure as means ± standard error of the mean (SEM). Data were analyzed for significant differences between groups using a one-way analysis of variance and for significant differences within groups using Student's t-test. A P value less than 0.05 was considered to be significant.

Results

Bolus injections of ACh produced a decrease in PP after submaximal vasoconstriction with Phe (10^{-7} M). The decrease in PP induced by ACh was almost abolished in kidneys preserved with EC solution for 72 h. However, in kidneys preserved with EC containing nicardipine or UK 38485, the effect of ACh persisted, as shown in Fig. 1.

As has previously been reported from the same research laboratories [8], the vasodilator response to ACh was attenuated after the addition of MB to the perfusion medium (data not shown). Such an effect was observed in all of the experiments.

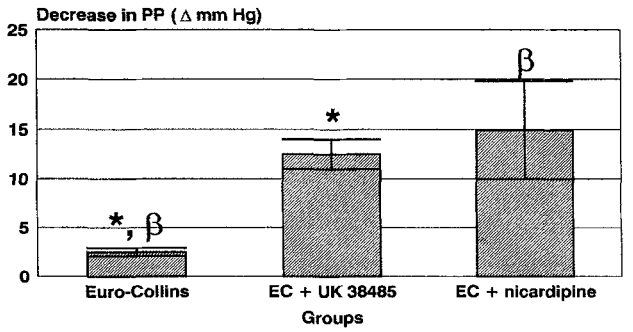

Fig. 1 Decrease in perfusion pressure (PP) induced by acetylcholine (ACh, 10^{-7} M) in kidneys preserved in Euro-Collins *(EC)* solution, EC + UK 38485, and EC + nicardipine for 72 h. Each *column* represents the mean value and *vertical bars* on the column show SEM *, β $P < 0.05$

Discussion

Prolonged hypothermic preservation of kidneys causes injury to the tissue, especially to the vascular endothelium, which can adversely affect the outcome of transplantation. This can lead to delayed graft function or even rejection.

In the present study, at the end of 72 h of hypothermic storage, the response of the kidney vascular endothelium to ACh, mediated by EDRF (NO), was still intact. Moreover, this vasodilator response was significantly augmented with the addition of either the thromboxane synthetase inhibitor or the calcium channel blocker compared to the EC solution alone. The results were taken as evidence of the cytoprotective effect of both the thromboxane synthetase inhibitor and the calcium channel blocker. After pretreatment with MB, the vasodilator effect of ACh was abolished. This effect is due to the inhibition of soluble guanylate cyclase by MB, preventing the production of c-GMP in vascular smooth muscle, which is the signal transduction mechanism of NO [14]. This shows that the arterial vasodilator activity of EDRF (NO) is mediated by the activation of guanylate cyclase [12, 16].

Increased cellular calcium may activate processes that lead to cellular injury and death. Calcium channel blockers have been shown to prevent ischemic injury by inhibiting the influx of Ca^{2+} through the cell membrane [17]. Moreover, calcium channel blockers can improve renal blood flow and ameliorate renal ischemic injury, as demonstrated in the present study [5, 21].

Strong evidence exists for the increased production of TxA_2, a potent vasoconstrictor and platelet aggrega-

tor, during both ischemic injury and allograft rejection [1, 7, 18]. TxA_2 plays an important role in the impairment of renal function and renal blood flow. The inhibition of TxA_2 synthesis with UK 38485 improved kidney vascular endothelial function after exposure to 72 h of cold ischemia. Moreover, shifting the balance between prostacyclin (PGI_2) and thromboxane A_2 towards PGI_2 may have some influence on the cytoprotective effect of the thromboxane synthetase inhibitor.

EDRF not only relaxes vascular smooth muscle and platelet adhesion, but is also a potent anti-aggregator substance [2]. Ischemic injury results in an alteration in the secretory function of the vascular endothelium and is responsible for the impairment of endothelium-dependent relaxation. In these circumstances, the endothelium loses its ability to prevent vasospasm, due to an inability to secrete EDRF.

We conclude that the capacity of kidney vascular endothelium to synthesize vasoactive substances that modulate the underlying smooth muscle is still intact after 72 h of hypothermic storage. The addition of the thromboxane synthetase inhibitor UK 38485 or of the calcium channel blocker nicardipine improves this secretory function significantly. Therefore, the addition of these cytoprotective substances to standard preservation solutions may be useful for improving post-transplant graft function.

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