

Endothelium-dependent inhibition of Na(+)-K+ ATPase activity in rabbit aorta by hyperglycemia. Possible role of endothelium-derived nitric oxide.

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Research Article

Hyperglycemia has been shown to diminish Na(+)-K+ ATPase activity in rabbit aorta. To examine the basis for this effect, aortic rings were incubated for 3 h in Krebs-Henseleit solution containing 5.5 or 44 mM glucose, and Na(+)-K+ ATPase activity was then quantified on the basis of ouabain-sensitive (OS) ⁸⁶Rb-uptake. Incubation with 44 mM glucose medium caused a 60% decrease in Na(+)-K+ ATPase activity in rings with intact endothelium (from 0.22 +/- 0.01 to 0.091 +/- 0.006 nmol/min per mg dry wt; P less than 0.01). Similar decreases (45%; P less than 0.01) in Na(+)-K+ ATPase activity were seen when rings incubated with 5.5 mM glucose were exposed to NG-monomethyl L-arginine (300 microM), an inhibitor of endothelium-derived nitric oxide (EDNO) synthesis or when the endothelium was removed (43% decrease). The decrease in Na(+)-K+ ATPase activity induced by hyperglycemia was totally reversed upon adding to the medium either L-arginine, a precursor of EDNO biosynthesis or sodium nitroprusside, which bypasses endothelium and directly activates the soluble guanylate cyclase in vascular smooth muscle. A decrease in Na(+)-K+ ATPase activity (42%; P less than 0.05), only seen in the presence of endothelium, was also observed in aortas taken directly from alloxan-induced diabetic rabbits. These studies suggest that the decrease in vascular Na(+)-K+ ATPase activity induced by hyperglycemia is related, at least in part, to a [...]

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Endothelium-dependent Inhibition of Na⁺-K⁺ ATPase Activity in Rabbit Aorta by Hyperglycemia

Possible Role of Endothelium-derived Nitric Oxide

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Abstract

Hyperglycemia has been shown to diminish Na⁺-K⁺ ATPase activity in rabbit aorta. To examine the basis for this effect, aortic rings were incubated for 3 h in Krebs-Henseleit solution containing 5.5 or 44 mM glucose, and Na⁺-K⁺ ATPase activity was then quantified on the basis of ouabain-sensitive (OS) ⁸⁶Rb-uptake. Incubation with 44 mM glucose medium caused a 60% decrease in Na⁺-K⁺ ATPase activity in rings with intact endothelium (from 0.22±0.01 to 0.091±0.006 nmol/min per mg dry wt; *P* < 0.01). Similar decreases (45%; *P* < 0.01) in Na⁺-K⁺ ATPase activity were seen when rings incubated with 5.5 mM glucose were exposed to N^G-monomethyl L-arginine (300 μM), an inhibitor of endothelium-derived nitric oxide (EDNO) synthesis or when the endothelium was removed (43% decrease). The decrease in Na⁺-K⁺ ATPase activity induced by hyperglycemia was totally reversed upon adding to the medium either L-arginine, a precursor of EDNO biosynthesis or sodium nitroprusside, which bypasses endothelium and directly activates the soluble guanylate cyclase in vascular smooth muscle. A decrease in Na⁺-K⁺ ATPase activity (42%; *P* < 0.05), only seen in the presence of endothelium, was also observed in aortas taken directly from alloxan-induced diabetic rabbits. These studies suggest that the decrease in vascular Na⁺-K⁺ ATPase activity induced by hyperglycemia is related, at least in part, to a decrease in the basal release of EDNO. They also suggest that alterations in basal EDNO release and possibly Na⁺-K⁺ ATPase activity contribute to the impairment in vascular relaxation caused by hyperglycemia and diabetes. (*J. Clin. Invest.* 1992. 90:727-732.) **Key words:** diabetes • ouabain • N^G-monomethyl L-arginine • cyclic GMP • prostaglandins

Introduction

Hyperglycemia alters the function of vascular tissues and may contribute to the development of both the micro- and macrovascular complications of diabetes (1). It has been suggested that hyperglycemia might exert such effects by inhibiting Na⁺-K⁺ ATPase activity (2). Na⁺-K⁺ ATPase maintains the cellular ionic milieu and by virtue of this, it is thought to influence

functions such as growth, differentiation, and contraction of vascular smooth muscle (3). In addition, derangements in the Na⁺-K⁺ ATPase activity have been implicated in the pathogenesis of hypertension (4). Hyperglycemia-induced decreases in Na⁺-K⁺ ATPase activity in nerve and vascular tissue have been related to a depletion of cellular myo-inositol, increased flux through the aldose reductase pathway, and an alteration in protein kinase C (PKC)¹ activity (5).

Vascular endothelial cells release a variety of factors that regulate vascular smooth muscle contraction and relaxation (6). Although the relationship of these factors to vascular Na⁺-K⁺ ATPase activity is unclear, it has recently been demonstrated that endothelium-dependent relaxation of the aorta is impaired at high glucose concentrations in vitro (7), and by diabetes in vivo (8). It has also been shown that the release of endothelium-derived factors is altered by hyperglycemia (7-10). In addition, endothelial cells have been demonstrated to stimulate Na⁺-K⁺ ATPase activity in co-cultured vascular smooth muscle cells (11). The present study explores whether the decrease in Na⁺-K⁺ ATPase activity caused by hyperglycemia in rabbit aorta (12), is related to altered synthesis and/or release of endothelium derived factor(s), and especially endothelium-derived nitric oxide (EDNO). In addition, we have examined whether a similar decrease in Na⁺-K⁺ ATPase activity occurs in the aorta as a consequence of diabetes.

Methods

Male New Zealand white rabbits (2.5-3.0 kg) were killed by exsanguination under pentobarbital (30 mg/kg, i.v.) anesthesia. In some studies, rabbits were rendered diabetic by the administration of alloxan (150 mg/kg i.v.) and were killed 6 wk later. The presence and severity of diabetes were ascertained on the basis of multiple blood glucose measurements and weight gain over the 6 wk (Table I). The thoracic aorta was quickly excised and placed in cold (4°C) physiological salt solution (PSS) containing (mM): 118 NaCl, 4.5 KCl, 0.54 MgCl₂, 2.5 CaCl₂, 1.2 NaH₂PO₄, 25.0 NaHCO₃, and 5.5 D-glucose. Rings (6-8 mm) were prepared after the aorta was dissected free of connective tissue with special care taken to avoid damage to the endothelium. Endothelium from the aortic rings was removed by inserting a stainless steel wire into the lumen and gently rolling the ring on a filter paper soaked in PSS. In selected rings, the presence or absence of endothelium was determined by the ability of acetylcholine (100 nM) to relax the ring when it was isometrically contracted with endothelin and by silver staining (13). Before the experiments, all normal or diabetic aortic rings were equilibrated for 1 h in PSS (37°C, pH 7.4) that was continuously gassed with 95% O₂ - 5% CO₂. Aortas incubated under these conditions for up to 5 h have been reported to have normal total

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1. *Abbreviations used in this paper:* EDNO, endothelium-derived nitric oxide; LNMMA, N^G-monomethyl L-arginine; OI, ouabain insensitive; OS, ouabain sensitive; PKC, protein kinase C; PSS, physiological salt solution; SNP, sodium nitroprusside.

fluid and extracellular volume (14). In addition, we have found that their rate of oxygen consumption ($6.1 \mu\text{mol}/\text{mg}$ wet wt per h; $n = 2$;) is identical to that reported for rabbit thoracic aorta by Chace and Odessey (14) and Simmons and co-workers (15).

Measurement of ^{86}Rb -uptake. ^{86}Rb -uptake by the aorta was determined as described previously (16). In brief, after the equilibration period, aortic rings were incubated in PSS (100 ml) containing normal (5.5 mM) or high (11, 22, or 44 mM) glucose concentrations for 3 h with the media exchanged hourly. When the aortas from diabetic rabbits were used, all incubations were performed in PSS containing 5.5 mM glucose. After the incubation, tissues were transferred to a vial containing 3 ml of PSS with normal or high glucose (37°C), in which they were gassed constantly with 95% O_2 - 5% CO_2 and incubated for another 10 min. At the end of this time, $^{86}\text{RbCl}$ ($2 \mu\text{Ci}/\text{ml}$) was added to the vial for 10 min. The tissues were next washed in cold (4°C) unlabeled PSS (80 ml) for 2 min in order to remove radioisotope from the extracellular compartment, blotted on a filter paper, and then placed in a plastic vial and dried overnight in an oven at 100°C. The $^{86}\text{Rb}^+$ content of tissue was determined by gamma counting. To determine ouabain-sensitive (OS) ^{86}Rb -uptake, the maximally effective concentration of ouabain (0.2 mM) was added 10 min before the introduction of $^{86}\text{RbCl}$ into the media. The OS portion of the ^{86}Rb -uptake, which is known to be an index of $\text{Na}^+ - \text{K}^+$ ATPase activity (17), was calculated by subtracting the ouabain-insensitive (OI) ^{86}Rb -uptake from the total ^{86}Rb -uptake. Results are expressed as nmol/min per mg dry tissue wt \pm SE and are based on an extracellular K^+ concentration of 4.5 mM in all experiments. Statistical evaluation of data was performed using unpaired *t* test (unless stated otherwise) with the minimum significance defined as $P < 0.05$. The incubation conditions were chosen on the basis of preliminary studies in which we compared the effects of preincubation for various periods of time up to 6 h on ^{86}Rb -uptake. The 3-h incubation period was selected because no significant differences in total, OS, or OI ^{86}Rb -uptake were observed between 1 and 3 h incubation in PSS. In contrast, total and OS ^{86}Rb -uptake were diminished by 15 and 50%, respectively, compared to the 3-h value, in endothelium-intact rings incubated in normal PSS for 6 h (data not shown).

Materials. N^G -monomethyl L-arginine acetate was purchased from Calbiochem Corp. (La Jolla, CA) and $^{86}\text{RbCl}$ from New England Nuclear (Boston, MA). All other drugs and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Results

Effect of hyperglycemia and hyperosmolarity on aortic $\text{Na}^+ - \text{K}^+$ ATPase activity. Aortic segments with intact endothelium were incubated for 3 h in PSS containing either 5.5, 11, 22, or 44 mM glucose. As shown in Fig. 1 A, OS ^{86}Rb -uptake ($\text{Na}^+ - \text{K}^+$ ATPase activity) was diminished by 47 and 60%, respectively, in aortas incubated at the two higher glucose concentrations. The effect of hyperglycemia was also time dependent, as 1 h of incubation with 44 mM glucose did not alter OS ^{86}Rb -uptake by endothelium-intact rings (data not shown). In contrast to these findings, OI ^{86}Rb -uptake, which comprised $\sim 70\%$ of total ^{86}Rb -uptake at 5.5 mM glucose, was not altered significantly in rings incubated with the higher glucose concentrations (Fig. 1 B). No alteration in OS ^{86}Rb -uptake (0.18 ± 0.02 nmol/min per mg; $n = 4$) occurred when aortas were incubated with 38.5 mM mannitol in the presence of 5.5 mM glucose, suggesting that the decrease in uptake observed at the high glucose concentrations was not due to hyperosmolarity.

Role of endothelium in mediating the decrease in $\text{Na}^+ - \text{K}^+$ ATPase activity caused by hyperglycemia. Since hyperglycemia alters the release of a number of endothelium-derived factors by the aorta and by endothelial cells in culture (7, 9, 10), some

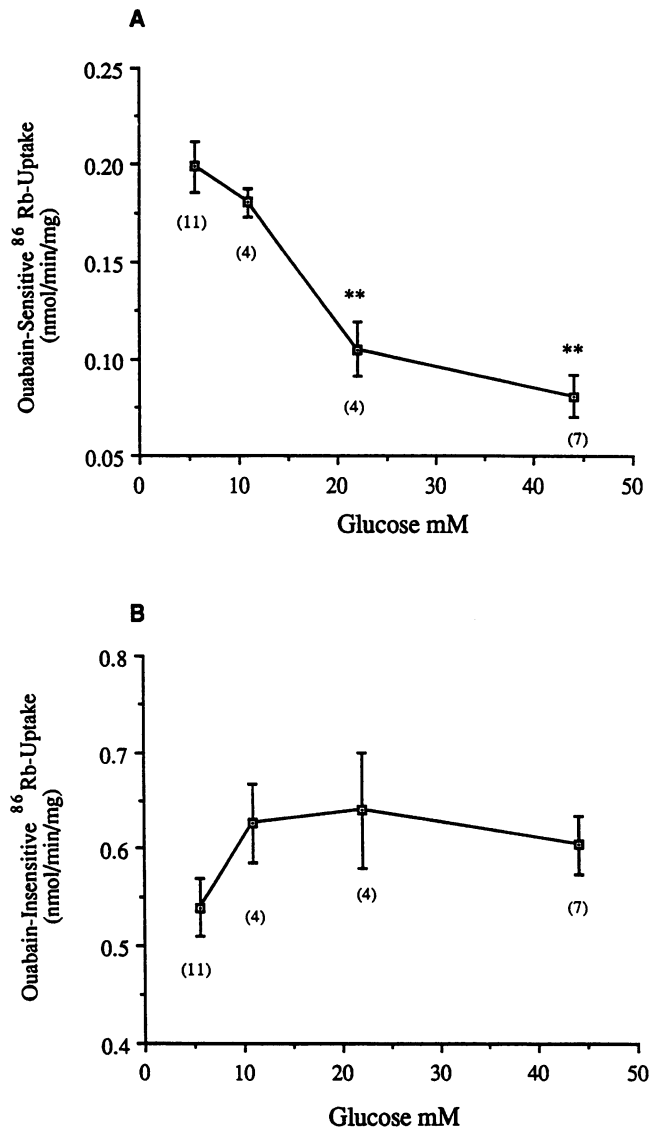


Figure 1. Concentration-dependent inhibition of ouabain-sensitive (OS) ^{86}Rb -uptake by glucose. Effect of glucose concentrations after 3 h incubation on OS ^{86}Rb -uptake (A) and ouabain-insensitive ^{86}Rb -uptake (B) was studied in endothelium-intact rings. Data are means \pm SE with numbers of experiments in parentheses. **Values significantly different from that of rings incubated in 5.5 mM glucose, $P < 0.01$.

of the initial studies were repeated using aortic rings from which the endothelium had been removed. As shown in Fig. 2, removal of endothelium resulted in a 43% decrease in OS ^{86}Rb -uptake in aortic segments incubated at 5.5 mM glucose. No further decrease in OS ^{86}Rb -uptake was observed when the medium contained 44 mM glucose, indicating that the effects of hyperglycemia and endothelial denudation are not additive.

Uptake in endothelial cells did not significantly contribute to OS ^{86}Rb -uptake by an intact aorta, since OS ^{86}Rb -uptake (0.23 ± 0.024 nmol/min per mg dry wt; $n = 4$) in rings from which endothelium was removed after the completion of ^{86}Rb -uptake in 5.5 mM glucose, was not different from that of rings with an intact endothelium (0.22 ± 0.01 nmol/min per mg). OI ^{86}Rb -uptake was not altered by removal of the endothelium at either 5.5 or 44 mM glucose (data not shown).

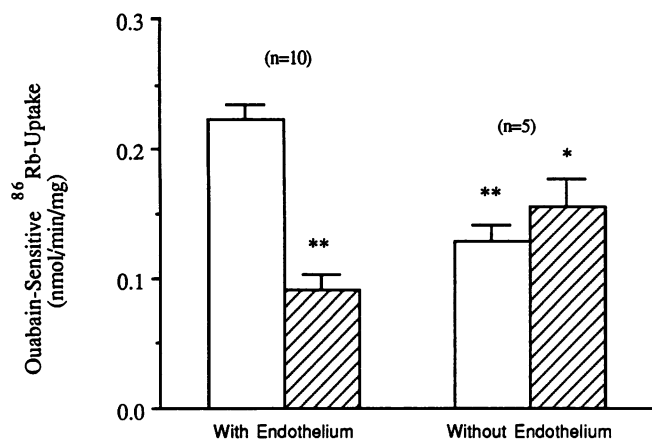


Figure 2. Endothelium-dependent inhibition of OS ^{86}Rb -uptake by hyperglycemia. Aortic rings with or without endothelium were incubated for 3 h in PSS containing 5.5 or 44 mM glucose. At the end of the incubation, OS ^{86}Rb -uptake was determined as described in Methods. Results are means \pm SE with number of experiments in parentheses. *, **Values significantly different from that of aorta with endothelium incubated in 5.5 mM glucose, $P < 0.05$ and $P < 0.01$, respectively. □, 5.5 mM glucose; ■, 44 mM glucose.

Effect of N^G -monomethyl L-arginine. To assess if alterations in the release or synthesis of EDNO could contribute to the inhibition of $\text{Na}^+ - \text{K}^+$ ATPase activity induced by hyperglycemia, OS ^{86}Rb -uptake was determined in aortic rings incubated with N^G -monomethyl L-arginine (LNMMA), an inhibitor of EDNO biosynthesis (18, 19). As shown in Fig. 3, exposure of endothelium-intact rings during the last 30 min of the incubation to 0.3 mM LNMMA caused a 45% decrease in OS ^{86}Rb -uptake (Fig. 3). In contrast, LNMMA did not add to the inhibition of OS ^{86}Rb -uptake caused by either incubation at 44 mM glucose (Fig. 3) or removal of the endothelium (data not shown). In addition, it did not alter OI ^{86}Rb -uptake under any of these conditions (data not shown).

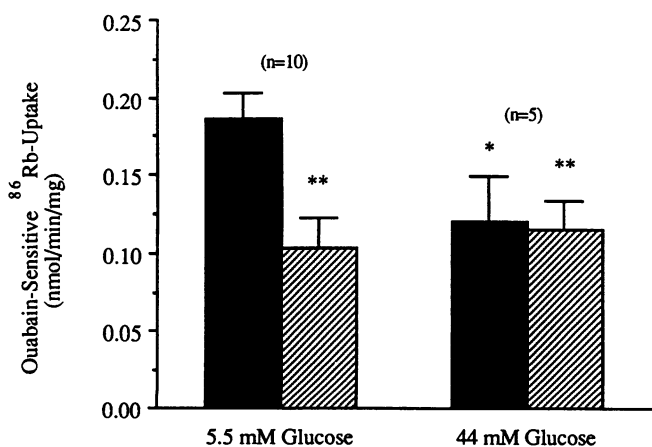


Figure 3. Effect of LNMMA on OS ^{86}Rb -uptake by endothelium-intact aortic rings. LNMMA (0.3 mM) was present for the final 30 min of a 3-h incubation at the indicated glucose concentrations. Data are means \pm SE with number of observations indicated in parentheses. **Values significantly different from that of a control aorta incubated with 5.5 mM glucose, $P < 0.05$ and $P < 0.01$, respectively. ■, Control; ▨, LNMMA.

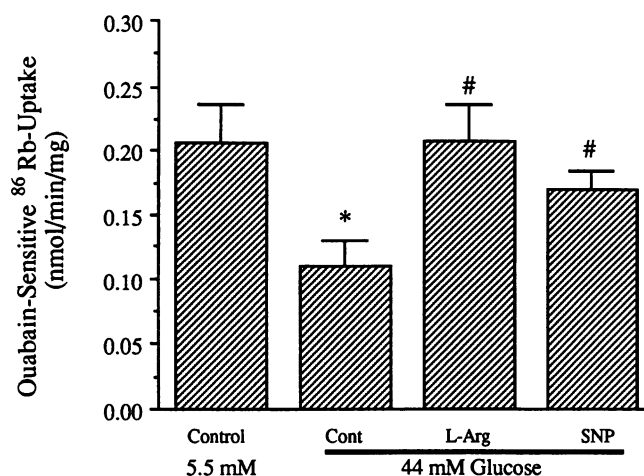


Figure 4. Reversal of hyperglycemia-induced inhibition of OS ^{86}Rb -uptake by L-arginine and sodium nitroprusside (SNP) in endothelium-intact aorta. L-arginine (0.3 mM) and SNP (10 μM) were added to the incubation media during the final 30 and 10 min, respectively, of the 3-h incubation. When L-arginine (0.3 mM) was present in the media during the entire 3-h incubation, hyperglycemia failed to decrease OS ^{86}Rb -uptake. Results are means \pm SE of five experiments. *Values significantly different from that of aorta incubated in 5.5 or 44 mM glucose, $P < 0.05$.

Effects of L-arginine and sodium nitroprusside. To gain further insight into the role of EDNO in modulating $\text{Na}^+ - \text{K}^+$ ATPase activity, the effect of hyperglycemia on OS ^{86}Rb -uptake was examined in the presence of L-arginine or sodium nitroprusside (SNP). L-arginine is a biosynthetic precursor of EDNO (18, 19) and it has been shown to correct the impairment of endothelium-dependent responses in atherosclerotic arteries and cerebral vessels from hypercholesterolemic rabbits (20, 21). SNP is metabolized into nitric oxide (NO), which in turn activates soluble guanylate cyclase in vascular smooth muscle (22, 23). The addition to the medium of L-arginine (0.3 mM) during the final 30 min of the study reversed the inhibition of OS ^{86}Rb -uptake observed in rings incubated with 44 mM glucose (Fig. 4). Likewise, the addition of L-arginine at the beginning of the 3-h incubation prevented the decrease in OS ^{86}Rb -uptake caused by 44 mM glucose (0.20 nmol/min per mg; $n = 2$). In contrast, incubation for 30 min or 3 h with L-arginine had no effect on OS ^{86}Rb -uptake in aortic rings incubated at 5.5 mM glucose (data not shown). Incubation with SNP (10 μM) for 10 min also reversed the inhibition of OS ^{86}Rb -uptake induced by hyperglycemia (Fig. 4). SNP did not significantly alter OS ^{86}Rb -uptake in endothelium-intact aortic rings incubated with 5.5 mM glucose (control = 0.21 ± 0.03 nmol/min per mg vs SNP = 0.23 ± 0.04 nmol/min per mg; $n = 3$). SNP did increase OS ^{86}Rb -uptake in aortic rings from which endothelium had been removed before incubation by ~80% (control = 0.14 ± 0.01 nmol/min per mg; SNP = 0.25 ± 0.03 nmol/min per mg; $n = 4$, $P < 0.05$), to levels not significantly different from OS ^{86}Rb -uptake by endothelium-intact aortic rings in 5.5 mM glucose.

Prostaglandins and $\text{Na}^+ - \text{K}^+$ ATPase activity. Hyperglycemia has been reported to increase the release of vasoconstrictive prostanoids from rabbit aortic rings (7, 9). Addition of the cyclooxygenase inhibitor indomethacin, at a concentration that has been shown to inhibit the formation of prostanoids

and prevent hyperglycemia-induced inhibition of endothelium-dependent responses in rabbit aorta (7), had no effect on OS ^{86}Rb -uptake in rings incubated with either 5.5 or 44 mM glucose (Fig. 5).

Effect of diabetes on ouabain-sensitive ^{86}Rb -uptake. To determine if the inhibition of $\text{Na}^+\text{-K}^+$ ATPase activity caused by hyperglycemia in vitro also occurs in diabetic animals in vivo, OS ^{86}Rb -uptake was measured in aortic rings from alloxan-diabetic rabbits. Rabbits had been made diabetic 6 wk previously and were characterized on the basis of multiple blood glucose measurements and weight gain. Rabbits in whom blood glucose levels were consistently > 250 mg/dl and weight gain over 6 wk was < 0.5 g, were classified as severely diabetic. Other rabbits with hyperglycemia were classified as mildly diabetic.

As shown in Table I, OS ^{86}Rb -uptake was decreased by 42% in endothelium-intact rings from severely diabetic rabbits incubated with 5.5 mM glucose, compared with rings from control animals. In contrast, only a 5% decrease in OS ^{86}Rb -uptake, which was not statistically significant, occurred in the mildly diabetic group (Table I). Removal of endothelium caused a significant decrease in OS ^{86}Rb -uptake in rings from control rabbits in agreement with the findings in Fig. 2. With the endothelium removed, no difference was observed between OS ^{86}Rb -uptake in the control and mildly diabetic groups and the two severely diabetic rabbits (Table I). In contrast to OS ^{86}Rb -uptake, OI ^{86}Rb -uptake was not decreased in the diabetic group; instead in the mildly diabetic rabbits, it was moderately increased in the absence of endothelium (Table I).

Discussion

The results suggest that hyperglycemia depresses $\text{Na}^+\text{-K}^+$ ATPase activity in the aorta by inhibiting EDNO synthesis under basal conditions. They also suggest that the basal release of EDNO contributes to the maintenance of the $\text{Na}^+\text{-K}^+$ ATPase activity in vascular smooth muscle. That the decrease in $\text{Na}^+\text{-K}^+$ ATPase activity caused by hyperglycemia was related to altered synthesis and release of EDNO is supported by a num-

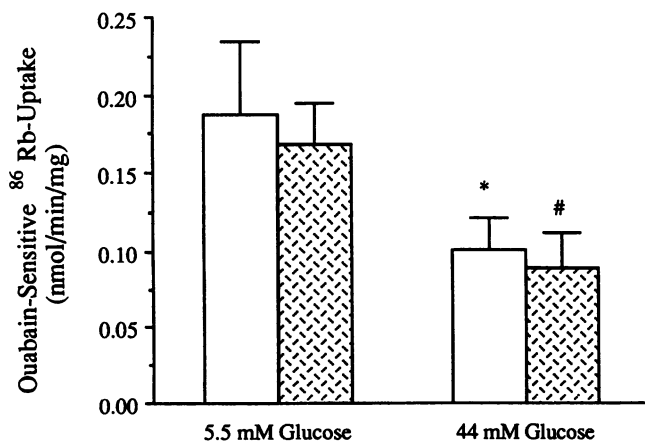


Figure 5. Lack of effect of cyclooxygenase inhibitor indomethacin on hyperglycemia-induced inhibition of OS ^{86}Rb -uptake. Indomethacin (10 μM) was added to the incubation media during the final 30 min of a 3-h incubation in 5.5 or 44 mM glucose. Data are means \pm SE of five experiments. *,# Values significantly different from those of a control aorta incubated with 5.5 mM glucose and indomethacin, respectively, $P < 0.05$. □, Control; ▣, indomethacin.

ber of findings: it is seen only in rings with an intact endothelium; it is mimicked by incubation with LNMMA, an inhibitor of EDNO synthesis; it is prevented and reversed by the addition of L-arginine, a precursor of EDNO, to the incubation media; and the effects of hyperglycemia and LNMMA on $\text{Na}^+\text{-K}^+$ ATPase activity were not additive. That LNMMA does not have a direct effect on vascular smooth muscle is suggested by the observation that its effect and the effect of endothelium removal on OS ^{86}Rb -uptake were not additive.

A role for continuous basal formation of EDNO in the regulation of blood pressure and vascular tone has been demonstrated (24, 25). EDNO is believed to cause vascular relaxation by increasing the content of cGMP in vascular smooth muscle (26). In keeping with such role, in the present study SNP, which directly releases NO and stimulates guanylate cyclase by an endothelium-independent mechanism (22, 23), reverses the inhibition of $\text{Na}^+\text{-K}^+$ ATPase activity caused both by hyperglycemia (Fig. 4) or removal of the endothelium. Others have proposed that an endothelium-dependent increase in cGMP levels stimulates $\text{Na}^+\text{-K}^+$ ATPase activity and that $\text{Na}^+\text{-K}^+$ ATPase plays a role in endothelium-dependent relaxation of the aorta (27, 28). Whether stimulation of $\text{Na}^+\text{-K}^+$ ATPase activity in the aorta by NO is due to an increase in intracellular Na^+ levels, phosphorylation of Na^+ -pump or some other mechanism remains to be determined.

L-arginine had no effect on basal $\text{Na}^+\text{-K}^+$ ATPase activity in aortic rings incubated with 5.5 mM glucose. The observation that it both prevents and reverses the decrease in $\text{Na}^+\text{-K}^+$ ATPase activity caused by hyperglycemia raises two possibilities: first, hyperglycemia may decrease the affinity of NO synthase for L-arginine, or, secondly, it could diminish NO synthesis by causing arginine depletion. We think that the latter is unlikely for a number of reasons. First, exogenous arginine has been shown to affect vascular function under conditions when there is ample arginine available in the extracellular pool. Thus Rositch et al. (21) have recently shown that L-arginine normalizes impaired endothelium-dependent vascular responses in the cerebral vessels from hypercholesterolemic rabbits and Cooke et al. (20) have demonstrated a similar finding in thoracic aortas of these rabbits. Secondly, arginine depletion in a bovine inter-pulmonary artery preparation (a threefold decrease) has been reported, but only after it is incubated at 37°C for 24 h and intermittently exposed to A23187 (29). Although these comparisons make it seem unlikely that cell arginine pool is depleted in an intact aorta incubated for 3 h with glucose, further studies are needed to confirm this conclusion.

SNP increased $\text{Na}^+\text{-K}^+$ ATPase activity in endothelium-denuded aortic rings and in rings incubated with glucose-enriched medium but not in endothelium-intact rings incubated with 5.5 mM glucose (see Results and Fig. 4). The sensitivity to SNP has been reported to be diminished in vessels with intact endothelium (30). It is believed that this could relate to an effect of basal release of EDNO to desensitize the underlying smooth muscle to exogenous nitrovasodilators (30, 31). In keeping with such a notion, removal of endothelium has been shown to augment the response to SNP and sodium nitrite in rat aorta (30).

The data also indicate that the inhibition of $\text{Na}^+\text{-K}^+$ ATPase activity caused by hyperglycemia under basal conditions is not mediated by prostaglandins. The cyclooxygenase inhibitor, indomethacin, did not prevent or reverse hyperglycemia-mediated inhibition of $\text{Na}^+\text{-K}^+$ ATPase activity. Likewise, it had no effect on the basal rate of OS ^{86}Rb -uptake (Fig. 5). Tesfa-

Table I. Effect of Diabetes on OS and OI ⁸⁶Rb-Uptake in Rabbit Aorta

Group	Weight gain kg	Blood glucose mg/dl	⁸⁶ Rb-Uptake	
			Ouabain-sensitive nmol/min per mg dry wt	Ouabain-insensitive nmol/min per mg dry wt
With endothelium				
Control	0.91±0.15 (6)	93±5 (4)	0.198±0.01 (6)	0.504±0.026 (6)
Diabetic				
Mild	0.69±0.10 (3)	213±4 (3)	0.189±0.04 (3)	0.575±0.049 (3)
Severe	0.10±0.11 (3)	443±18 (3)	0.124±0.03 (3)*	0.445±0.024 (3)
Without endothelium				
Control	0.91±0.15 (6)	93±5 (4)	0.125±0.02 (6)*	0.514±0.035 (6)
Diabetic				
Mild	0.69±0.10 (3)	213±4 (3)	0.148±0.024 (3)	0.62±0.057 (3)†
Severe	0.10±0.11 (3)	443±18 (3)	0.152 (2)	0.357 (2)

Rabbits (initial weight ~2.3 kg) were rendered diabetic 6 wk previously and characterized as mild or severely diabetic, as described in Methods and Results. OS ⁸⁶Rb-uptake by endothelium-intact and -denuded rings was determined in the presence of 5.5 mM glucose. Control rabbits were studied on the same day. Data are expressed as means±SE with numbers of observations in parentheses. *, † Values significantly different from controls with or without endothelium, respectively, *P* < 0.05.

marium et al. have observed impaired endothelium-dependent relaxation and an increased generation of vasoconstrictor prostanooids from endothelium, when abdominal aortas incubated in a hyperglycemic milieu are stimulated by acetylcholine (7). Similar findings were observed in abdominal aortas from diabetic rabbits (8). Our data provide no evidence that these prostanooids inhibit Na⁺-K⁺ ATPase activity under basal conditions. The release of another endothelium-derived vasoactive factor, endothelin (32), has been shown to be altered by hyperglycemia and by diabetes (10, 33). We have recently reported that endothelin activates Na⁺-K⁺ ATPase activity in endothelium-intact rabbit aorta (16). The possibilities that hyperglycemia may affect the release of endothelin or its ability to stimulate Na⁺-K⁺ ATPase activity in rabbit aorta remain to be evaluated.

Hyperglycemia has been shown to decrease Na⁺-K⁺ ATPase activity in cultured endothelial cells (34). However, such an effect does not account for the decrease in Na⁺-K⁺ ATPase activity hyperglycemia causes in incubated aortic rings (Figs. 1 and 2). Thus removal of endothelium after 10 min incubation with ⁸⁶RbCl had no effect on the magnitude of OS ⁸⁶Rb-uptake, indicating that the contribution of the endothelium is small. This is not unexpected since the smooth muscle to endothelial cell layer ratio in the aorta is ~ 30:1. In addition, in cultured bovine aortic endothelial cells, O'Donnell has shown that only ~ 11% of total ⁸⁶Rb-uptake can be accounted for as OS ⁸⁶Rb-uptake (35).

Hyperglycemia increases the de novo synthesis of diacylglycerol in cultured endothelial cells and in microvessels (34, 36) and it activates PKC in cultured endothelium (35). PKC activation in arteries by direct acting phorbol esters and by vasoconstrictive agents is associated with an increase in Na⁺-K⁺ ATPase activity in the smooth muscle (16, 37). Therefore the inhibition of Na⁺-K⁺ ATPase activity by hyperglycemia is unlikely due to a direct effect of PKC activation in the smooth muscle. A direct activation of PKC by phorbol esters has been shown to inhibit the release of EDNO from cultured porcine aortic endothelial cells (38), and from isolated canine coronary and femoral arteries (39). Thus, PKC has been proposed to play a role in the synthesis and release of EDNO. In keeping

with such a notion, the impairment of endothelium-dependent relaxation by hyperglycemia appears to be mimicked by pretreatment with a phorbol ester and it is prevented by an inhibitor of PKC in rabbit aorta (40). A possible explanation for our findings is that hyperglycemia, by increasing PKC activity in endothelial cells, inhibits the synthesis and release of EDNO and this secondarily diminishes Na⁺-K⁺ ATPase activity in vascular smooth muscle.

An increase in aldose reductase activity and depletion of cellular myo-inositol pools has also been shown in tissues in which Na⁺-K⁺ ATPase activity is diminished by hyperglycemia (5, 12). Simmons and Winegrad (41) have recently suggested that hyperglycemia specifically inhibits an adenosine-regulated Na⁺-K⁺ ATPase. The role of these pathways in EDNO synthesis or release remains to be determined.

The effect of diabetes on Na⁺-K⁺ ATPase activity in the aorta appeared to be similar to that of hyperglycemia. An inhibition of Na⁺-K⁺ ATPase activity was seen in endothelium-intact aortas from severely diabetic rabbits. After removal of the endothelium, this inhibition was no longer observed in rings incubated in 5.5 mM glucose. Aortas from diabetic rabbits, like aortas incubated in an hyperglycemic medium (7), demonstrate impaired endothelium-dependent relaxation (8). Also diminished cGMP levels have been observed in aortas of diabetic rabbits and rats (42, 43). These results suggest that the inhibition of aortic Na⁺-K⁺ ATPase activity caused by diabetes, like that caused by hyperglycemia, is related to diminished EDNO synthesis and release. A decrease in the release of EDNO has recently been proposed to occur in diabetes (44, 45). In addition, quenching of EDNO by advanced glycosylation end-products in experimental diabetes has been described (46).

In conclusion, the results suggest that hyperglycemia is associated with a decrease in the basal synthesis and release of endothelium-derived NO in rabbit aorta, that leads to a decrease in Na⁺-K⁺ ATPase activity. Whether these changes contribute to the altered vascular reactivity reported in diabetes and to the increased propensity of patients with diabetes to hypertension, angina, and ischemic vascular disease remains to be determined.

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