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Role of Na^+-K^+ Pump on Endothelium-dependent Relaxation

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= ABSTRACT=

To study the underlying mechanism through which the endothelium-dependent relaxation is inhibited by blocking the Na^+-K^+ pump, the effects of Na^+-K^+ pump blockade on the release of EDRF and its relaxing activity were examined, using organ bath study, bioassay technique, and cGMP measurement. Endothelium-dependent relaxation was attenuated by blocking the Na^+-K^+ pump in the vascular ring with intact endothelium. In bioassay experiment, EDRF release was decreased with the blockade of the Na^+-K^+ pump in the EDRF donor strip. Endothelium-dependent increase of cGMP level was suppressed by inhibiting the Na^+-K^+ pump in the test strips. The magnitude of relaxation of test strip which was induced by the perfusate that had passed through the EDRF donor strip was decreased with the blockade of the Na^+-K^+ pump in the test strip. Therefore, it could be suggested that the attenuation of endothelium-dependent relaxation caused by inhibiting Na^+-K^+ pump activity is due to both the decreased release of EDRF from endothelial cells and the decreased sensitivity of the smooth muscle cells to EDRF.

Key Words: Na^+-K^+ pump, vascular smooth muscle, endothelium, EDRF release, the sensitivity of vascular smooth muscle to EDRF.

Index terms: Na^+-K^+ pump, vascular smooth muscle, endothelium, EDRF release, the sensitivity of vascular smooth muscle to EDRF

INTRODUCTION

Recently endogenous digitalis has been considered as one of the causes evoking hypertension. Endogenous digitalis-like substance was found in the blood of a patient with low-renin hypertension and acute volume expansion. It was observed that the capacity of plasma to inhibit the Na^+-K^+ pump was raised in hereditary and acquired forms of hypertension (Haddy & Overbeck, 1976; Haddy et al, 1979; Poston et al, 1981; Hamlyn et al, 1982; Gray et al, 1986; Millett et al, 1986; Holland et al, 1987). Now an endogenous ouabain-like compound has been isolated from human plasma (Ludens et al, 1991) and it is similar to plant-derived ouabain in its molecular structure (Mathews et al, 1991) and biochemical action (Hamyln et al, 1989).

It was also reported that there was endothelial dysfunction or decreased sensitivity of vascular smooth muscle cell to EDRF in the hypertensive animals (Van de Voorde & Leusen, 1986; Luscher et al, 1991). It is also

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well-known that not only endothelium-dependent relaxation is attenuated and but also endothelium-dependent increase of cGMP level in vascular smooth muscle cell is suppressed with the blockade of the Na^+-K^+ pump (Rapoport et al, 1985). However, the effect of Na^+-K^+ pump blockade on EDRF release and its relaxing activity has not been determined yet. Thus, the present study was undertaken to investigate the effects of Na^+-K^+ pump inhibitors on EDRF release and its relaxing activity.

MATERIALS AND METHODS

Tissues

Rabbits of either sex, weighing about 2 kg, were killed by exsanguination from the femoral artery under sodium pentothal anesthesia. The thoracic aorta and common carotid artery were excised and immersed in the physiological salt solution at room temperature and cleaned by removing connective tissues surrounding the vessel. The endothelial cells were removed mechanically using the method of Furchgott and Zawadzki[15], i.e., the internal surface of the vessel was rubbed gently by a moistened cotton ball.

All experiments were carried out at the temperature of 37℃ and 10^{-5} M indomethacin pretreatment was carried out 5 min prior to the start of the experiment to eliminate the effect of PG I_2.

Organ bath study

Mechanical responses were recorded from ring segments (1.5~2.0 mm in width) of the thoracic aorta, using a pair of L-shaped fine glass rods; one was connected to a three dimensional manipulator and the other was connected to the mechano-transducer (Harvard). The muscle ring was mounted between these glass rods and suspenden in a water-jacketed Lucite chamber of 0.5 ml capacity and perfused with Krebs solution, at a constant flow rate of 4 ml/min, using a peristaltic pump (Pharmacia Fine Chemicals). Isometric contractions of these muscle preparations were displayed on a pen-writing recorder (Gould RS 3200).

Bioassay experiment

A long aortic segment including thoracic and abdominal aorta with endothelium (EDRF donor aorta, about 5 cm in length) was cannulated with glass cannulas (ID 1.8 mm) and placed in the organ chamber filled with modified Krebs-Ringer solution which was aerated with 95% O_2-5% CO_2 mixed gas and kept at 37℃. The aortic segment was perfused at a constant speed (2 ml/min) by means of a roller pump (Pharmacia Fine Chemicals) with modified Krebs-Ringer solution. A transverse strip of thoracic aorta or a ring of carotid artery, from which the endothelium had been removed (bioassay test strip), was suspended directly below the organ chamber by means of two glass stirrups and superfused with the perfusate that had passed through the aortic segment. One stirrup was connected to an isometric force transducer (Harvard of Gould J 968) and changes in isometric tension were recorded

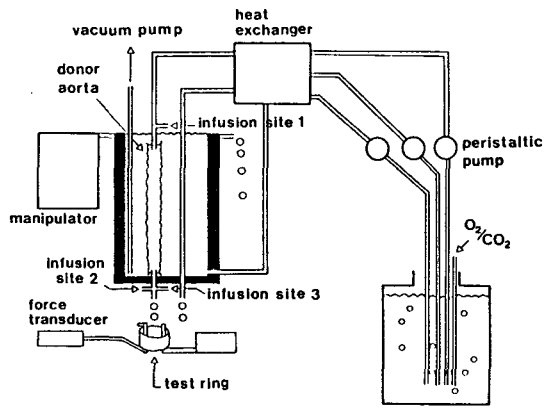

Fig. 1. A schematic representation of the bioassay apparatus. A aortic transverse strip or carotid arterial ring without endothelium was mounted for isometric tension recording and superfused with Krebs-Ringer bicarbonate solution that had passed through the thoracic aortic segment with endothelium.

(Gould RS 3200). The assembly of the organ chamber could be moved freely above the bioassay test strip using micromanipulator (Fig. 1).

The bioassay strip was superfused with the solution that had passed through the EDRF-donor aortic segment for 60 min. Thereafter, it was stretched until the basal tension reached approximately 2 g. After 2 hours, norepinephrine (NE) was infused through the infusion site (IS) 2 with an infusion pump (Scientific & Research Instruments) at a rate of 0.02 ml/min (to reach 10^{-7} or $5\times^{-5}$ M final perfusate concentration). When the contraction reached a steady state, acetylcholine (ACh) was infused through infusion site 1 by means of an infusion pump at a rate of 0.02 ml/min (to reach 10^{-6} M final perfusate concentration).

cGMP measurement

Rubbed or unrubbed carotid arterial rings were prepared as described above and suspended in the Lucite chamber described above. At any time in the experiment, the chamber could be instantly dropped down using the micromanipulator, leaving the supported preparation accessible for immediate freezing with a stainless plate cooled in liquid N_2. The muscle strip was contracted with NE in the presence of indomethacin and ACh was used to evoke endothelium-dependent response. When the strip was relaxed almost completely, the chember was instantly dropped down and the strip was immediately frozen. The Cyclic GMP level was assayed as previously described by Katsuki and Murad (1977). Briefly, frozen tissues were homogenized in 6% trichloroacetic acid and the samples were centrifuged. Supernatant fractions were extracted with ether and radioimmunoassayed for cGMP (NEN) (Kimura et al, 1974; Steiner et al, 1972). The amount of protein was determined by the method of Lowry et al (1951) using bovine serum albumin as standard.

Solutions

The ionic composition of modified Krebs solution was as follows (in mM); Na^+ 137.4, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, HCO_3^- 1,2, HCO_3^- 25, $H_2PO_4^-$ 1.2, Cl^- 134, calcium disodium ethylendiaminetetraacetic acid 0.026, glucose 11.5. The solution was aerated with O_2 containing 5% CO_2, and the pH of the solution was maintained at 7.3～7.4.

Drugs

Drugs used were acetylcholine chloride, indomethacin, l-norepinephrine HCl, and ouabain (all from Sigma, U.S.A.).

Statistics

Experimental values were expressed by the means±standard deviation (S.D.) with n= number of observations. Statistical significances were determined using Student t-test, and probabilities of less than 5% ($p<0.05$) were considered significant.

RESULT

Effect of Na^+-K^+ pump blocking on endothelium-dependent relaxation

The left panel of Figure 2 shows the effect of ouabain on ACh-induced relaxation. The strips with intact endothelium were pre-exposed to ouabain-containing solution for 15 min. The magnitude of relaxation was decreased by ouabain in a dose dependent manner. The right panel showes the effect of K^+-free solution on endothelium-dependent relaxation. In K^+-free solution, endothelium-dependent relaxation was attenuated. In order to make clear the inhibitory effect of Na^+-K^+ pump blocking on endothelium-dependent relaxation, the effect of Na^+-K^+ pump blockade of endothelial cells on EDRF release and/or that of Na^+-K^+ pump blocking in vascular smooth muscle cells on the relaxing activity of EDRF were examined.

Effect of ouabain on the relaxing activity of EDRF

The result of the bioassay experiments are

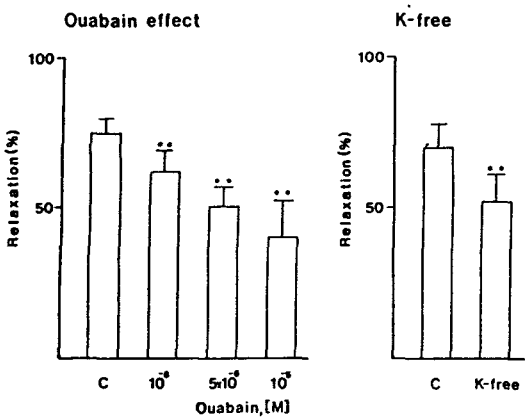

*Fig. 2. Effects of ouabain or K^+-free solution on endothelium-dependent relaxation in the strips of thoracic aorta. The strips with endothelium were pretreated with ouabain or K^+-free solution for 15 min. The magnitudes of endothelium-dependent relaxations were significantly decreased by treatment with ouabain or K^+-free solution. Results are shown as means±S.D. ($n=10$ in each case) and are expressed as percent relaxation of the NE contraction. *$p<0.05$ as compared with the control group. C, control.*

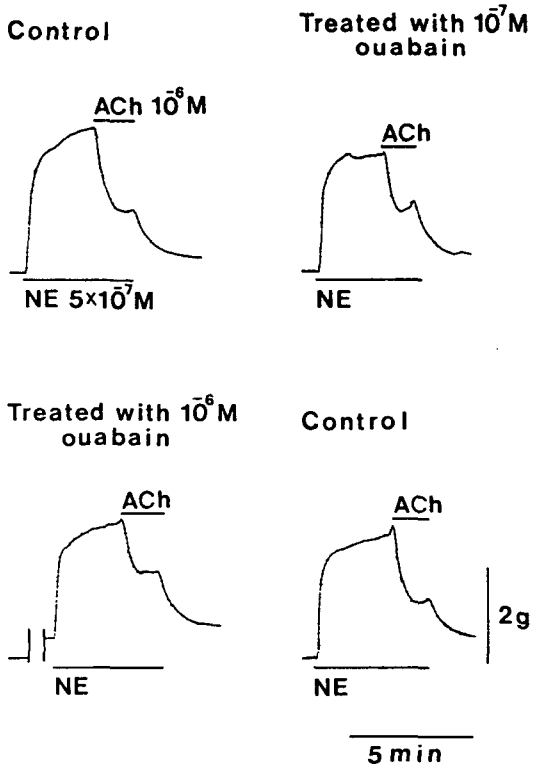

Fig. 3. Effect of ouabain on the relaxing activity of the perfusate in the smooth muscles of carotid arterial ring. The test ring was pretreated with 10^{-7}, 10^{-6} M ouabain for 30 min by infusing throught infusion site 3. The magnitudes of relaxations of test strips were slightly decreased by the pretreatment, and the decrease was enhanced with increase of ouabain concentration.

shown in Figure 3 and 4, where the test ring was superfused with the perfusate that had passed through the EDRF donor aortic segment. In this condition, ouabain was infused at site 2 for 30 min. Then, NE was infused at site 3. When ACh was infused at site 1, we could observe the effect of EDRF released from the donor aorta. Through this bioassay technique, we could differentiate the effect of ouabain on the test strip selectively. After such an experiment, we repeated the bioassay study every 20 min without the infusion of ouabain.

Through treatment with 10^{-7} or 10^{-6} M ouabain, basal tone was slightly increased, and the relaxation of the test strip was atteunated in a dose dependent manner, which was not remarkable.

When the test ring was pretreated with 10^{-5} M ouabain, basal tone was increased markedly. The magnitude of relaxation of the test strip induced by the perfusate that had passed through the EDRF donor aorta was decreased. Therefore, it could be suggested that the effect of the perfusate on the contraction by ouabain and NE was diminished. However, the effect of the perfusate on the contraction by NE was not comparable with that of the previous experiment shown in Figure 3, since the increase in basal tone caused by ouabain was great and the additional contraction by NE was small (Fig. 4). In the experiments done every 20 min after this experiment was finished (Fig. 4), the magnitudes of relaxation 20 and 40 min after the

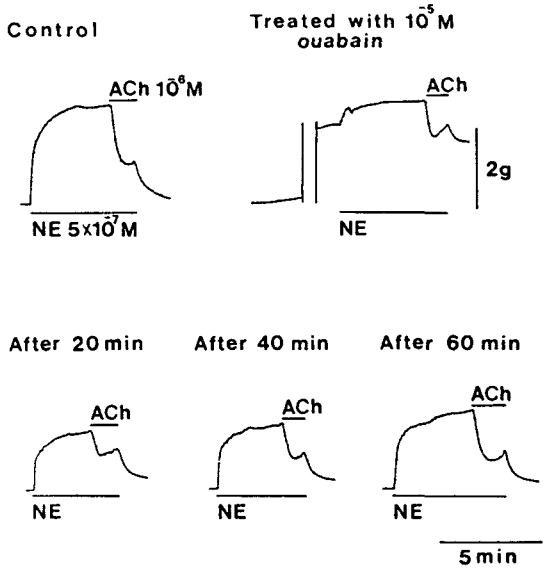

Fig. 4. Effect of ouabain on the relaxing activity of perfusate in the smooth muscles of carotid artery strip. The test ring was pretreated with 10^{-5} M ouabain for 30 min by infusing through IS 3. The basal tone of the test strip was markedly increased and the magnitude of relaxation of test strip induced by the perfusate was decreased (upper panel). Note that the decreased sensitivity of the test strip to the perfusate had not recovered 40 min after stopping ouabain treatment and the magnitude of relaxation of the test strip was recovered almost to the control value 60 min after stopping ouabain treatment (lower panel).

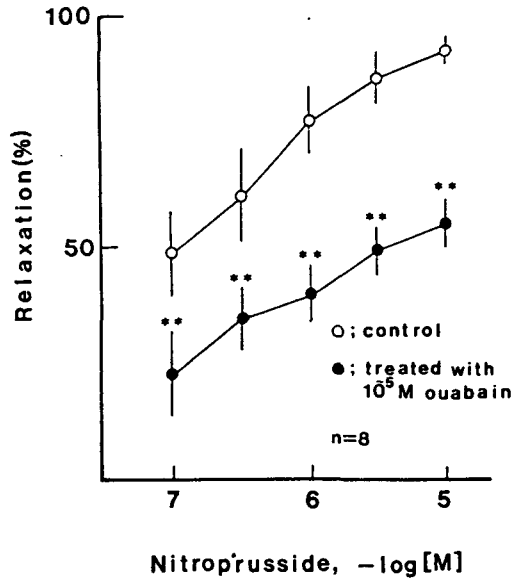

Fig. 5. Effect of ouabain on the endothelium-independent relaxation induced by nitroprusside. The strips were pretreated with ouabain for 30 min. The magnitude of relaxation induced by nitroprusside was decreased by pretreatment with ouabain. Results are shown as means ± S.D. and are expressed as percent relaxation of the contraction induced by 3×10^{-7} M NE. $^{**}p<0.01$ as compared with the control group.

cessation of ouabain infusion were still less than the control value. The magnitude of relaxation recovered almost to the control value 60 min after the ouabain infusion ended. These results suggest that the relaxing activity of EDRF is decreased by blocking Na^+-K^+ pump. The result shown in Fig. 5 also suggests that the relaxing activity of EDRF is decreased by blocking the Na^+-K^+ pump, since the magnitude of relaxation by sodium nitroprusside. Therefore, it can be suggested that the sensitivity of vasular smooth muscle to EDRF is decreased by blocking the Na^+-K^+ pump.

The effect of Na^+-K^+ pump blocking on EDRF release from endothelial cells

Bioassay experiment: In the experiment of Figure 6 the test strip was dripped with the solution passing through polyethylene tubing to the test strip. The manipulator allowed us to choose the solutions dripping onto the test strip. 10 uM of ouabain was infused through site 1. After the pretreatment with ouabain of the EDRF donor aorta for 30 min, ouabain infusion was stopped. We switched the solution dripping to the test strip to the perfusate that had passed through EDRF donor aorta, using the manipulator.

Figure 6 shows the result. The magnitude of

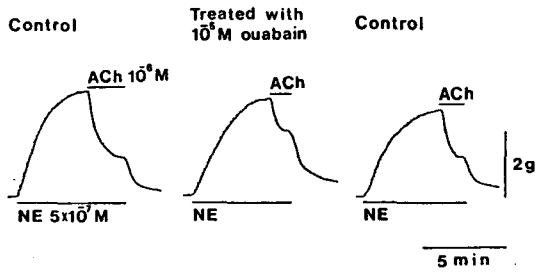

Fig. 6. Effect of ouabain on the release of EDRF (s) from the thoracic aortic segment in bioassay experiment. Selective pretreatment of the EDRF donor aorta with ouabain for 30 minutes reduced the magnitude of relaxation induced by perfusate.

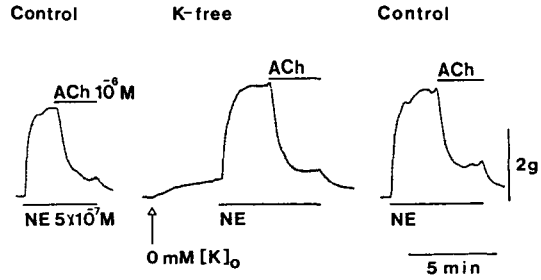

Fig. 8. Effect of K^+-free solution on the release of EDRF (s) from the thoracic aortic segment in bioassay experiment. Blockade of the Na^+-K^+ pump of the EDRF donor aorta with K^+-free solution for 5 minutes did not decrease the magnitude of relaxation of the test strip by the perfusate.

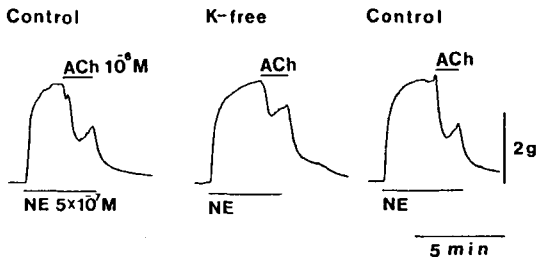

Fig. 7. Effect of K^+-free solution on the release of EDRF (s) from the thoracic aortic segment in bioassay experiment. Selective blockade of the Na^+-K^+ pump of the EDRF donor aorta with K^+-free solution for 30 minutes decreased the magnitude of relaxation of the test strip by the perfusate. This result was similar to that previously shown in Fig. 6, and these results suggest that EDRF release from endothelial cells is decreasd by blockade of the Na^+-K^+ pump.

relaxation profuced by the perfusate was decreased by the pretreatment with ouabain, in comparis to those shown in the experiments before and after the treatment with ouabain.

In the experiment whose result was shown in the Figure 7, K^+-free Krebs-Ringer solution was used for the solutions perfusing inside and outside of the donor aorta, and K^+ was added into the persufate that had passed through the EDRF donor aorta by infusing K^+ through infusion site 2 in order to normalize K^+ concentration (6 mM) of the perfusate. Thus, in this experimental condition, the EDRF donor aorta was in the K^+-free condition, whereas the test ring was in normal K^+ concentration.

Figure 7 shows the result. The magnitude of relaxation produced by the perfusate was decreased by K^+ depletion inside and outside the donor aorta, compared with those shown in the experiments before and after the treatment with ouabain.

The experimental arrangement of the experiment shown in Figure 8 was similar to the previous experiment, except that K^+ was not infused to normalize the K^+ concentration of the perfusate. Thus, in this experimental condition, both the EDRF donor aorta and the test strip were in K^+-free conditions. The period of incubation with K^+-free solution was 5 min. Basal tone of the test strip was increased, which seems to be the result of Na^+-K^+ pump blocking. On the other hand, the magnitude of relaxation was not changed.

Cyclic GMP levels: Endothelium-dependent increase of cGMP in vascular smooth muscle cell was suppressed by pretreatment with 10^{-5} M ouabain or by incubation in K^+-free solu-

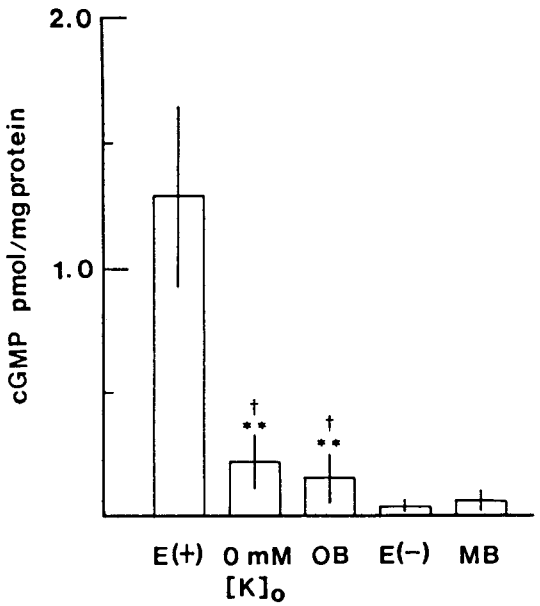

*Fig. 9. Effects of ouabain or K^+-free solution on cGMP level in the vascular smooth muscle cell. ** means significant difference between control and rings treated with ouabain (10^{-5} M) or K^+-free solution ($p<0.01$) and † means significant difference between rings treated with ouabain (10^{-5} M) or K^+-free solution and rings treated with MB (10^{-5} M) or endothelium-removed rings ($p<0.05$). E (+), endothelium intact; E (−), endothelium removed; OB, ouabain; MB, methylene blue.*

tion, for 30 min (Fig. 9). These data are well consistent with the ones previously presented, which suggest that EDRF release is suppressed by the inhibition of the Na^+-K^+ pump.

These results suggest that EDRF release from endothelial cells by acetylcholine is suppressed, but not completely, by Na^+-K^+ pump blocking.

DISCUSSION

The present study demonstracted that the inhibitory effect of Na^+-K^+ pump blocking on endothelium-dependent relaxation to ACh was due, in part, to the decreased sensitivity of vascular smooth muscle to EDRF and, in part, to the decreased EDRF release from endothelial cells.

The mechanism(s) by which the sensitivity of vascular smooth muscle cells to EDRF is decreased by blocking the Na^+-K^+ pump is not known. Wheter the decreased sensitivity is due to partial inhibition of soluble guanylate cyclase activation by EDRF and/or to increased hydrolysis of cyclic GMP and/or to the decreased activity of cyclic GMP and protein kinase G is not clear. However the decreased sensitivity does not appear to be due to increased hydrolysis, since exposure to a relatively specific cGMP phosphodiesterase inhibitor, M&B 22,948, did not alter the magnitude of inhibition (Rapoport et al, 1985).

NE-evoked responses are augmented by blocking the Na^+-K^+ pump and the augmented contractile responses are probably due to the increased availability of Ca^{2+} to activate contraction, because the inhibition of the Na^+-K^+ pump raises intracellular Na^{2+} concentration, and this then leads to an increase in intracellular Ca^{2+} concentration as a result of Na^+/Ca^{2+} exchange (Bova et al, 1990). In Fig. 8, the relaxing activity of EDRF was not decreased by the pretreatment with K^+-free solution for 5 min whereas basal tone of the test ring was being increased, which suggested that the Na^+-K^+ pump was blocked and intracellular Ca^{2+} was being increased. This result suggests that the decreased sensitivity of vascular smooth muscle to EDRF is not due to the Na^+-K^+ pump itself but due to intracellular changes caused by Na^+-K^+ pump blocking. Thus, the decreased sensitivity of vascular smooth muscle cells to EDRF may be due to the increase in intracellular Ca^{2+} concentration resulted from the reverse mode of action of Na^+/Ca^{2+} exchange.

The mechanism(s) by which the release of EDRF is decreased by blocking Na^+-K^+ pump is not known. One of the possible mechanism (s) is depolarization of endothelial cell membrane potential. From the facts that EDRF release is dependent on extracellular Ca^{2+} concentration (Griffith et al, 1986) and the voltage-op-

erated Ca^{2+} channel (VOC) is absent from the endothelial cell membrane (Coldman-Stanfield et al, 1987; Nilius & Riemann, 1990; Takeda et al, 1987) it could be supposed that the Ca^{2+} entered the cell through channels other than VOC (i.e., non-selective cation channel, receptor-operated Ca^{2+} channel, etc) in response to EDRF releasing agents, and the Ca^{2+} influx through these channels is decreased by depolarization of membrane potential, since, as a functional result, the driving force for Ca^{2+} is decreased with the depolarization. Therefore, the decreased EDRF release may be due to the decreased Ca^{2+} influx in endothelial cells as a result of depolarization. However there is a evidence that the inhibitory effect of aa^{+}-K^{+} pump blocking on endothelium-dependent relaxation (unpublished data) and EDRF release is dependent on the duration of Na^{+}-K^{+} pump blocking. More than 15 min of Na^{+}-K^{+} pump blocking is necessary to inhibit endothleium-dependent relaxation by ACh (unpublished data). In Figure 8, the magnitude of relaxation of the test ring is shown to be not decreased by pretreatment with K^{+}-free solution for 5 min although the basal tone of the test ring was increased, which suggested Na^{+}-K^{+} pump was blocked and intracellular Ca^{2+} concentration was increasing via Na^{+}-Ca^{+} exchange. This result suggest that EDRF release is not inhibited by the treatment. In contrast, some depolarization of the smooth muscle cells (Fleming, 1980) or cultured endothelial cells (Daut et al, 1988) occurred rapidly after the treatment with ouabain and K^{+}-free solution. Thus the decreased EDRF release does not appear to be due to depolarization. Another possible mechanism is the increased availability of Ca^{2+} to release EDRF, since inhibition of the Na^{+}-K^{+} pump raises intracellular Na^{2+}, and this then leads to an increase in intracellular Ca^{2+} as a result of Na^{+}/Ca^{2+} exchange. It is possible that the increase of intracellular Ca^{2+} facilitates EDRF release and depletes EDRF storage or EDRF precusors. Thus ACh-induced EDRF release may be decreased with the depletion. Such a view has received apparent support from Winguist et al (1985). These workers showed that the Na^{+}/Ca^{2+} exchange mechanism might play a role in the release of EDRF. However, there is no evidence that Na^{+}-K^{+} pump blocking stimulates EDRF release. Rapoport et al (1985) reported that cGMP level ofs rat thoracic aorta in the presence of NE was not changed by the treatment with ouabain and was decreased in K^{+}-free solution.

The indirect nature of the present evidence makes it difficult to formulate a conclusion regarding the mechanisms by which Na^{+}-K^{+} pump inhibitors decreases the sensitivity of vascular smooth muscle cells to EDRF as well as ACh-induced EDRF release from endothelial cells and it remains to be elucidated how the sensitivity of vascular smooth muscle cells to EDRF and the release of EDRF from endothelial cells are decreased with the Na^{+}-K^{+} pump blocking.

REFERENCES

Bova S, Goldman WF, Yuan X-J & Blaustein MP (1990) Influnce of the Na^{+} gradient on Ca^{2+} transients and contraction in vascular smooth muscle. *Am J Physiol* **259,** H409-H423

Coldman-Stanfield M, Schilling WP, Ritchie AK, Eskin SG, Navarro LT & Kunze DL (1987) Bradykinin-induced increases in cytosolic calcium and ionic currents in cultured bovine aortic endothelial cells. *Circ Res* **61,** 632-640

Daut J, Mehrke G, Nees S & Newman WH (1988) Passive electrical properties and electrogenic sodium transport of cultured guinea-pig coronary endothelial cells. *J Physiol* **402,** 237-254

Fleming WW (1980) The electrogenic N^{+}, K^{+} pump in smooth muscle. Physiologic and pharmacological significance. *Ann Rev Pharmacol Toxicol* **20,** 129-149

Furchgott RF & Zawadzki JV (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **228,** 373-376

Gray HH, Hilton PJ & Richardson RJ (1986) Effect of serum from patients with essential

hypertension on sodium transport in normal leukocytes. *Clin Sci* **70,** 583-586

Griffith TM, Edwards DH, Lewis MJ, Newby AC & Henderson AH (1986) Production of endothelium-derived relaxant factor is dependent on oxidative phosphorylation and extracellular calcium. *Cardiovasc Res* **20,** 7-12

Haddy FS & Overbeck HW (1976) The role of humoral agents in volume expanded hypertension. *Life Sci* **19,** 935-948

Haddy FJ, Pamnani MB & Clough DL (1979) Humoral factors and the sodium-potassium pump in volumn expanded hypertension. *Life Sci* **24,** 2105-2118

Hamyln JM, Harris DW & Ludens JH (1989) Digitalis-like activity in human plasma: Purification, affinity, and mechanism. *J Biol Chem* **264,** 7395-7404

Hamlyn JM, Ringel R, Schaeffer J, Levinson PD, Hamilton BP, Kowarski AA & Blaustein MP (1982) A circulating inhibitor of (Na^+-K^+) ATPase associated with essential hypertension. *Nature* **300,** 650-652

Holland S, Millett J, Alaghband-Zadeh J, de Wardener HE, Ferrari P & Bianchi G (1987) Cytochemically assayable Na-K-ATPase inhibition by Milan hypertensive rat plasma. *Hypertension* **9,** 498-503

Katsuki S & Murad F (1977) Regulation of adenosine cyclic 3′,5′-monophosphate levels and contractility in bovine trachial smooth muscle. *Mol Pharmacol* **13,** 330-341

Kimura H, Thomas E & Murad F (1974) Effect of decapitation, ether and pentobarbital on guanosine 3′,5′-phosphate and adenosine 3′,5′-phosphate levels in rat tissues. *Biochem Biophys Acta* **343,** 519-528

Lowry OH, Rosebrough NJ, Farr AL & Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* **193,** 265-275

Ludens JH, Clark MA, DuCharme DW, Harris DW, Lutzke BS, Mandel F, Mathews WR, Sutter DM & Hamlyn JM (1991) Purification of an endogenous digitalis-like factor from human plasma for structural analysis. *Hypertension* **17,** 923-929

Luscher TF, Vanhoutte PM, Boulanger C, Dohi Y & Buhler FR (1991) Endothelium dysfunction in hypertension. In *Cardiovascular significance of endothelium-derived vasoactive factors.* ed. Rubanyi GM New York, Futura Publishing Company, Inc. p 199-221

Mathews WR, DuCharme DW, Hamlyn JM, Harris DW, Mandel F, Clark MA & Ludens JH (1991) Mass spectral characterization of an endogenous digitalislike factor from human plasma. *Hypertension* **17,** 930-935

Millett JA, Holland SM, Alaghband-Zadeh J & de Wardener HE (1986) Na-K-ATPase-inhibiting and glucose-6-phosphate dehydrogenase-stimulating activity of plasma and hypothalamus of the Okamoto spontaneously hypertensive rat. *J Endocrinol* **108,** 69-73

Nilius B & Riemann D (1990) Ionic channels in human endothelial cells. *Gen Physiol Biophys* **9,** 89-112

Poston L, Sewell RB, Wilkinson SP, Richardson PJ, Williams R, Clarkson EM, MacGregor GA & de Wardener HE (1981) Evidence for a circulating sodium transport inhibitor in essential hypertension. *Brit Med J* **282,** 847-849

Rapoport RM, Schwartz K & Murad F (1985) Effects of Na^+-K^+ pump inhibitors and membrane depolarizing agents on acetylcholine-induced endothelium-dependent relaxation and cyclic GMP accumulation in rat aorta. *Eur J Pharmacol* **110,** 203-209

Steiner AL, Parker CW & Kipnis DM (1972) Radioimmunoassay for cyclic nucleotides. *J Biol Chem* **247,** 1106-1113

Takeda K, Schini V & Stoeckel H (1987) Voltage-activated potassium, but not calcium current in cultured bovine aortic endothelial cells. *Pflugers Arch* **410,** 385-393

Van de Voorde & Leusen I (1986) Endothelium-dependent and independent relaxation of aortic rings from hypertensive rats. *Am J Physiol* **250,** H711-H717

Winquist RJ, Bunting PB & Schofield TL (1985) Blockade of endothelium-dependent relaxation by the amiloride analog dichlorobenzamil: Possible role of Na^+/Ca^{++} exchange in the release of endothelium-derived relaxing factor. *J Pharmacol Exp Ther* **235,** 644-650