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Interaction between norepinephrine and angiotensin Ⅱ on Na^+ uptake in primary cultured rabbit renal proximal tubule cells

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초대배양한 토끼 신장 근위세뇨관세포의 Na^+ uptake에 대한 norepinephrine과 angiotensin Ⅱ의 상호작용

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초 록 : 신장 근위세뇨관세포들은 사구체에서 여과된 물질의 재흡수, 분비 및 대사에 관여하는 여러 호르몬들의 수용체들을 가지고 있다. 이들중에서 norepinephrine(NE)과 angiotensin Ⅱ(ANG Ⅱ)는 Na^+/H^+ 상호운반계를 조절함으로써 혈압조절에 관여하는 것으로 알려져 있으나 이들의 상호관계에 대해선 연구보고가 많지 않다. 본 연구는 초대배양한 토끼 신장 근위세뇨관세포를 이용한 Na^+ uptake 실험을 통하여 NE이 어떠한 수용체를 통하여 Na^+/H^+ 상호운반계를 조절하는지 그리고 이러한 작용에 있어서 NE과 ANG Ⅱ의 상호관계를 알아보고자 실시하였다.

NE($> 10^{-9}$M)은 Na^+ uptake를 유의성 있게 증가시켰다(10^{-9}M NE : 27±4% increase vs. Control; $p < 0.05$). α 길항제(phentolamine, 10^{-10}M)는 NE(10^{-9}M)에 의해 유도된 Na^+ uptake를 유의성 있게 차단하였으나(phentolamine+NE : 29±5% inhibition vs. NE ; $p < 0.05$), α_1(pra-zosin, 10^{-10}M) 및 α_2 길항제(yohimbine, 10^{-10}M)는 부분적으로 차단하였다. β 길항제(propra-nolol, 10^{-10}M)도 역시 NE에 의해 유도된 Na^+ uptake를 유의성 있게 차단하였으나(propranolol+NE : 24±6% inhibition vs. NE ; $p < 0.05$), β_1(atenolol, 10^{-10}M) 및 β_2 길항제(butoxamine, 10^{-10}M)는 부분적으로 차단하였다. 이러한 결과들은 NE에 의해 유도된 Na^+ uptake 증가작용은 $\alpha(\alpha_1$ 및 $\alpha_2)$와 $\beta(\beta_1$ 및 $\beta_2)$ 수용체 모두를 통하여 일어난다는 것을 시사해주고 있다.

ANG Ⅱ(10^{-11}M) 또는 NE(α_1, α_2, β_1, β_2 작동제) 단독처리군의 Na^+ uptake는 대조군에 비해

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유의성 있게 증가하였으나(ANG Ⅱ : 23±9% increase vs. Control ; $p < 0.05$), 병합처리시 상승작용은 나타나지 않았다. α 또는 β 길항제 처리시 NE 및 ANG Ⅱ에 의해 유도되었던 Na^+ uptake 증가는 유의성 있게 차단되었다(phentolamine+NE+ANG Ⅱ : 25±3% inhibition, propranolol+NE+ANG Ⅱ : 24±6% inhibition vs. NE+ANG Ⅱ, respectively ; $p < 0.05$). 이 결과들은 Na^+ uptake에 있어서 α(α_1 및 α_2)와 β(β_1 및 β_2) 수용체와 ANG Ⅱ의 관련성을 시사해 준다.

결론적으로 토끼 신장 근위세뇨관세포에서 NE은 α_1, α_2, β_1 및 β_2 수용체를 통하여 Na^+ uptake를 증가시켰으며 이들 수용체는 ANG Ⅱ의 Na^+ uptake 증가작용에 관여하였다.

Key words : Kidney, norepinephrine, angiotensin Ⅱ, Na^+ uptake.

Introduction

It is generally accepted that catecholamines act as antinatriuretic substances[1,2]. When renal adrenergic nerves were stimulated, renal excretion of sodium was decreased. In contrast, a decrease in renal nerve activity led to an increase in sodium excretion. It was shown that mammalian renal tubules have catecholamine receptors that directly modulate the transport of ions and water across the tubular cells[3,4]. The proximal tubules seem to be the main targets for catecholamine, which modulate their ability to reabsorb water and solute from urine[3,5~7].

Adrenergic receptors are classified into α receptors and β receptors. The former are generally subclassified into α_1 and α_2 and the latter into β_1 and β_2. These receptors are reported to be located in the kidney. α_1 and α_2 adrenergic receptors seem to be primarily localized to the renal cortex, with the highest concentration of binding sites in the proximal tubule[8,9]. β-adrenergic receptors also seem to be primarily localized to the renal cortex and the outer band of medullar[10,11]. Most of the evidence seems to suggest that enhanced tubule reabsorption of sodium, water, and other substances caused by renal nerve stimulation is effect through α_1-adrenoceptors[12]. In contrast, α_2-adrenoceptors stimulate or inhibit the fluid reabsorption[13~15]. In regard to β-adrenoceptors, β-adrenoceptor stimulation decrease or increase Na^+ reabsorption[16,17]. As described, the function of subtype of adrenoceptors on Na^+ transport is controversial and studies to date have not yet demonstrated functional effect by the subtype of both adrenoceptors in the same preparation.

In considering the critical role of the kidney in the maintenance of sodium homeostasis, the role of the renal nerves is integrated into the multiple complex control mechanisms that are involved ANG Ⅱ has been reported to increase Na^+ transport in proximal tubule cells[18]. Several lines of evidence suggested that there is an interaction between adrenergic receptor and ANG Ⅱ in some tissues. Renal sympathetic nerve stimulation increase renin secretion ratio and ANG Ⅱ generation[19,20]. In the innervated rat kidney administration of α_2 adrenoceptor agonist enhanced the response to ANG Ⅱ[21]. However, it is not yet clear whether there is an interaction between ANG Ⅱ and NE on Na^+ transport in the proximal tubule cells. Thus, to determine which subtype of adrenoceptors have an influence on Na^+ transport and furthermore, whether or not there is an interaction between NE and ANG Ⅱ on Na^+ transport, we conducted Na^+ uptake experiments in primary cultured rabbit renal proximal tubule cells(PTCs).

In this study, we found that NE stimulated Na^+ uptake via α_1, α_2, β_1, β_2 receptor and these receptors are involved in the ANG Ⅱ-induced stimulation of Na^+ uptake in the PTCs.

Materials and Methods

Materials : Male New Zealand White rabbits(1.5~2.0kg) were used for these experiments. Dulbecco's Modified Eagle's Medium, Nutrient Mixture F-12(Ham), Class Ⅳ collagenase and soybean trypsin inhibitor were purchased from Life Technologies(Grand Island, NY). Angiotensin Ⅱ(ANG Ⅱ), norepinephrine(NE), BSA fraction Ⅴ, hydrocortisone, insulin, ouabain, phenylephrine(α_1 agonist), clonidine(α_2 agonist), phentolamine(α antagonist), prazosin(α_1 antagonist), yohimbine(α_2 antagonist), dobutamine(β_1 agonist), terbutamine(β_2 agonist), propranolol(β antagonist), atenolol(β_1 antagonist), butoxamine(β_2 antagonist), and transferrin were purchased from the Sigma Company(St. Louis, Mo). $^{22}Na^+$ was purchased from Dupont/NEN.

All other reagents were of the highest purity commercially available. Liquiscint was obtained from National Diagnostics(Parsippany, NY). Iron oxide was prepared by the method of Cook and Pickering[22]. Stock solutions of iron oxide in 0.9% NaCl were sterilized using an autoclave and diluted with phosphate buffered saline prior to use.

Isolation of rabbit renal proximal tubules and culture conditions : Primary rabbit renal proximal tubule cell cultures were prepared by a modification of the method of Chung *et al*[23]. The basal medium, D-MEM/F-12, pH 7.4, was a 50 : 50 mixture of Dulbecco's Modified Eagle's Medium and Ham's F-12. The basal medium was further supplemented with 15mM HEPES buffer(pH 7.4) and 20mM sodium bicarbonate. Immediately prior to the use of the medium, three growth supplements(5μg/ml insulin, 5μg/ml transferrin, and 5×10^{-8}M hydrocortisone) were added. Water utilized in medium preparation was purified by means of a Milli Q deionization system. Kidneys of a male New Zealand White rabbit were perfused via the renal artery, first with PBS, and subsequently with D-MEM/F-12 containing 0.5% iron oxide(wt/vol) until the kidney turned grey-black in color. Renal cortical slices were prepared by cutting the renal cortex and then homogenized with 4 strokes of a sterile glass homogenizer. The homogenate was poured first through a 253μm and then a 83μm mesh filter. Tubules and glomeruli on top of the 83μm filter were transferred into sterile D-MEM/F-12 medium containing a magnetic stirring bar. Glomeruli(containing iron oxide) were removed with a magnetic stirring bar. The remaining proximal tubules were briefly incubated in D-MEM/F-12 containing 80μg/ml collagenase (Class Ⅳ) and 0.025% soybean trypsin inhibitor. The dissociated tubules were then washed by centrifugation, resuspended in D-MEM/F-12 containing the three supplements, and transferred into tissue culture dishes, PTCs were maintained at 37℃, in a 5% CO_2-humidified environment in D-MEM/F-12 medium containing the three supplements. Medium was changed one day after plating and every three days thereafter.

Na^+ uptake experiment : The confluent monolayers were incubated with NE or/and 10^{-11}M ANG Ⅱ with and without adrenoceptor agonists and antagonists for 4 hr before Na^+ uptake experiments. Uptake experiment was conducted as described by the method of Rindler *et al*[24]. For Na^+ uptake studies, the medium was removed by aspiration. Before the uptake period, the monolayers were washed twice with 100mM Tris-HCl buffer, pH 7.4. Na^+ uptake was measured at 37℃ for 30 min in an uptake buffer(10mM Tris, 1mM $CaCl_2$, 1mM $MgCl_2$, 140mM Choline chloride) containing 0.25μCi/ml $^{22}Na^+$ and 5×10^{-5}M ouabain(pH 7.4). At the end of the incubation period, the monolayers were gently washed three times with ice cold 100mM Tris-HCl buffer, pH 7.4 and the cells were solubilized with 1ml of 0.1% SDS. To determine the $^{22}Na^+$ incorporated intracellulary, 900μl of each sample was removed and counted in a liquid scintillation counter(Beckmann Co.). The remainder of each sample was used for protein determination[25]. The radioactivity counts in each sample were then normalized with respect to protein and were corrected for zero-time uptake per mg protein. All uptake measurements were made in triplicate.

Statistical analysis : Results were expressed as means± standard errors(S.E.). The difference between two mean values was analyzed by Student's *t*-test. The difference was considered statistically significant when $p < 0.05$.

Results

Effect of NE on Na^+ uptake : To determine the effect of NE on Na^+ uptake, the PTCs were treated with different

concentration of NE(10^{-9}, 10^{-6}, 10^{-4}M). As shown in Fig 1, the addition of NE in concentration of 10^{-9}, 10^{-6}, and 10^{-4}M to the PTCs for 4 hr resulted in the stimulation of Na^+ uptake by 27±4%, 20±10%, 32±10% as compared with control, respectively. 10^{-9}M NE which was close to physiological concentration was used in this experiment.

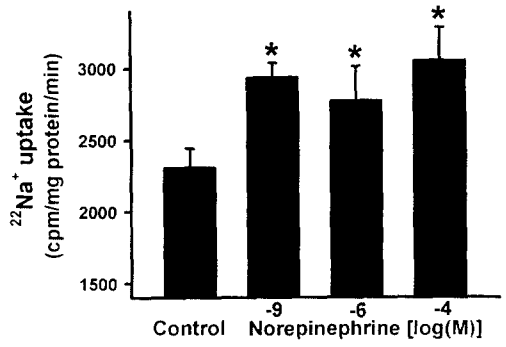

Fig 1. Effects of norepinephrine(NE) on Na^+ uptake. PTCs were incubated with different dose of NE(10^{-9}~10^{-4}M) for 4 hr. Values are the means±S.E. of 12 experiments performed on 4 different culutres. *p < 0.05 vs. Control.

Effect of α vs. β adrenoceptor antagonists on Na^+ uptake : To examine the adrenergic receptor subtype that mediates enhanced Na^+ uptake in response to NE, phentolamine(α antagonist, 10^{-10}M) and propranolol(β antagonist, 10^{-10}M) was employed. Phentolamine and propranolol inhibited the NE-induced stimulation of Na^+ uptake by 29± 5% and 24±6% compared with NE, respectively(Fig 2 ; p < 0.05). However, phentolamine and propranolol alone did not influence Na^+ uptake. These results demonstrate that NE enhance the Na^+ uptake into the PTCs, and their effects are mediated via both α- and β-adrenergic receptors.

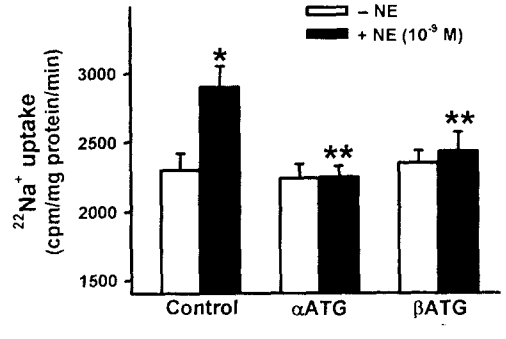

Fig 2. Effects of α- and β-adrenoceptor antagonist(ATG) on Na^+ uptake. PTCs were incubated with NE(10^{-9}M) for 4 hr after the preincubation of phentolamine(α ATG, 10^{-10}M) or propranolol (β ATG, 10^{-10}M) for 1 hr. Values are the means±S.E. of 9 experiments performed on 3 different cultures. *p < 0.05 vs. Control. **p < 0.05 vs. NE alone.

Effect of α_1 vs. α_2 adrenoceptor antagonists and agonists on Na^+ uptake : To further define the α receptor subtype in the NE-induced stimulation of Na^+ uptake, selective α_1 and α_2 antagonist were employed Fig 3 shows that prazosin(α_1 antagonist, 10^{-10}M) and yohimbine(α_2 antagonist, 10^{-10} M) partially blocked the NE-induced stimulation of Na^+ up-

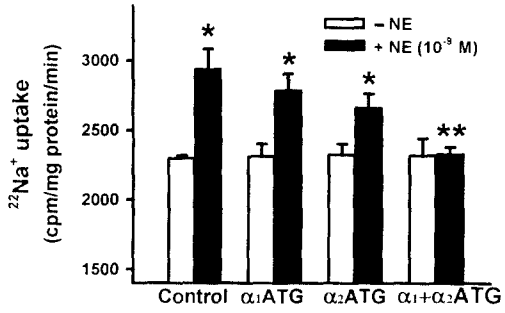

Fig 3. Effects of NE, α_1- and α_2-adrenoceptor antagonist(ATG) on Na^+ uptake. PTCs were incubated with NE(10^{-9}M) after the preincubation of vehicle, prazosin(α_1 ATG, 10^{-10}M), yohimbine(α_2 ATG, 10^{-10}M) alone or together for 1 hr. Values are the means±S.E. of 12 experiments performed on 4 different cultures. *p < 0.05 vs. Control. **p < 0.05 vs. NE alone.

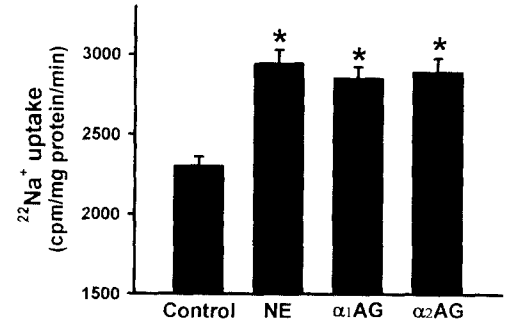

Fig 4. Effects of NE, α_1- and α_2-adrenoceptor agonist(AG) on Na^+ uptake. PTCs were incubated with NE(10^{-9}M), phenylephrine (α_1 AG, 10^{-9}M) or clonidine(α_2 AG, 10^{-9}M) for 4 hr. Values are the means±S.E. of 9 experiments performed on 3 different cultures. *p < 0.05 vs. Control.

take. But the coadministration of prazosin and yohimbine completely blocked NE-induced stimulation of Na^+ uptake (prazosin+yohimbine+NE : 27±4% inhibition vs. NE ; $p < 0.05$). These results indicate that the NE-induced stimulation of Na^+ uptake is mediated via both α_1 and α_2 receptors. To further establish the α adrenergic-mediated effect on Na^+ uptake, phenylephrine(α_1 agonist, 10^{-9}M) and clonidine(α_2 agonist, 10^{-9}M) was investigated. Phenylephrine and clonidine increased Na^+ uptake by 24±3% and 26±4%, respectively(Fig 4).

Effects of β_1 vs. β_2 adrenoceptor antagonists and agonists on Na^+ uptake : In addition to α receptor, to investigate the function of subtype of β receptors in NE-induced stimulation of Na^+ uptake, selective β_1 and β_2 antagonists were employed. As shown in Fig 5, atenolol(β_1 antagonist, 10^{-10} M) and butoxamine(β_2 antagonist, 10^{-10}M) partially blocked NE-induced stimulation of Na^+ uptake. However, atenolol+butoxamine blocked NE-induced stimulation of Na^+ uptake (atenolol+butoxamine+NE : 30±2% inhibition vs. NE ; $p < 0.05$). Atenolol or butoxamine did not inhibit the Na^+ uptake alone. Indeed, dobutamine(β_1 agonist, 10^{-9}M) and terbutaline(β_2 agonist, 10^{-9}M) increased Na^+ uptake by 17±2% and 27±8% as compared with control(Fig 6).

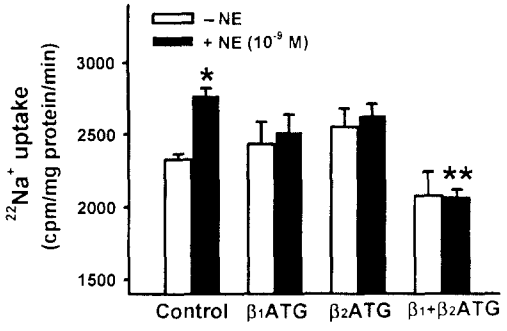

Fig 5. Effects of β_1- and β_2-adrenoceptor antagonist(ATG) on Na^+ uptake. PTCs were incubated with NE for 4 hr after the preincubation of vehicle, atenolol(β_1 ATG, 10^{-10}M) and butoxamine(β_2 ATG, 10^{-10}M) alone or together for 1 hr. $^*p < 0.05$ vs. Control. $^{**}p < 0.05$ vs. NE alone.

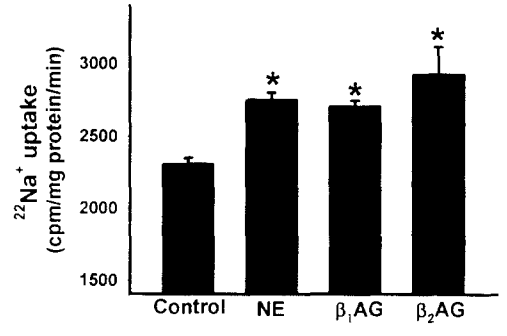

Fig 6. Effects of β_1- and β_2-adrenoceptor agonist(AG) on Na^+ uptake. PTCs were incubated with NE(10^{-9}M), dobutamine(β_1 AG, 10^{-9}M) and terbutamine(β_2 AG, 10^{-9}M). Values are the means±S.E. of 12 experiments performed on 4 different cultures. $^*p < 0.05$ vs. Control.

Interaction between NE and ANG Ⅱ on Na^+ uptake : As shown in Fig 7, ANG Ⅱ (10^{-11}M) also increased Na^+ uptake compared with control(ANG Ⅱ : 23±9% increase vs. Control ; $p < 0.05$). To examine the interaction between NE and ANG Ⅱ, NE(10^{-9}M) and ANG Ⅱ (10^{-11}M) were treated together. NE or ANG Ⅱ alone increased Na^+ uptake compared with control, while there was no synergistic effect on Na^+ uptake.

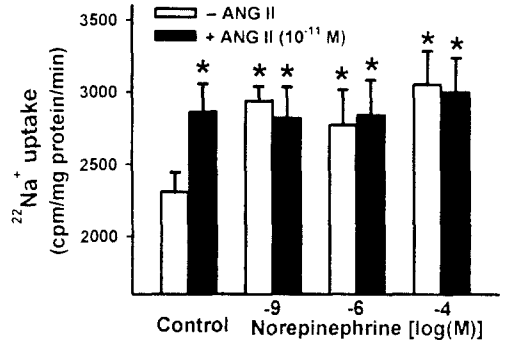

Fig 7. Effects of NE and ANG Ⅱ on Na^+ uptake. PTCs were incubated with ANG Ⅱ (10^{-11}M) alone or together with different dose of NE(10^{-9}~10^{-4}M) for 4 hr. Values are the means±S.E. of 12 experiments performed on 4 different cultures. $^*p < 0.05$ vs. Control.

To examine the interaction between the subtype of adrenergic receptor agonists and ANG Ⅱ, phenylephrine(α_1 agonist, 10^{-9}M), clonidine(α_2 agonist, 10^{-9}M), dobutamine(β_1 agonist, 10^{-9}M), or terbutaline(β_2 agonist, 10^{-9}M) was co-treated with ANG Ⅱ. Although respective agonist or ANG Ⅱ alone increased Na^+ uptake, there was also no synergistic effect(Fig 8, 10).

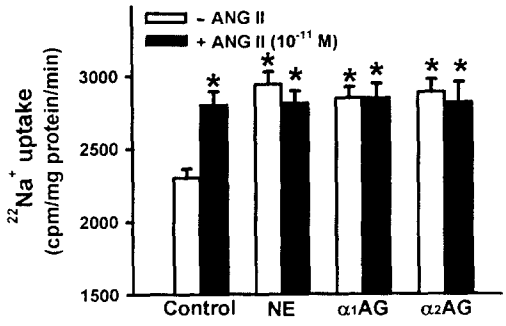

Fig 8. Effects of NE, α_1- or α_2-adrenoceptor agonist(AG) and ANG Ⅱ on Na^+ uptake. PTCs were incubated with ANG Ⅱ (10^{-11}M) alone or together with NE(10^{-9}M), phenylephrine(α_1 AG, 10^{-9}M) or clonidine(α_2 AG, 10^{-9}M) for 4 hr. Values are the means±S.E. of 12 experiments performed on 4 different cultures. *p < 0.05 vs. Control.

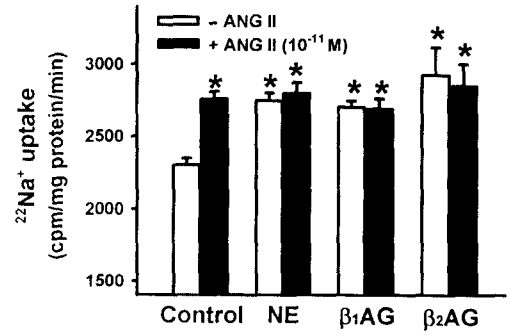

Fig 10. Effects of NE, β_1- or β_2-adrenoceptor agonist(AG) and ANG Ⅱ on Na^+ uptake. PTCs were incubated with ANG Ⅱ (10^{-11}M) alone or together with vehicle, NE(10^{-9}M), dobutamine (β_1 AG, 10^{-9}M) or terbutaline(β_2 AG, 10^{-9}M) for 4 hr. Values are the means±S.E. of 9 experiments performed on 3 different cultures. *p < 0.05 vs. Control.

When α_1 antagonist or α_2 antagonist was treated together with NE and ANG Ⅱ, NE- and ANG Ⅱ-induced stimulatory effect of Na^+ uptake was partially blocked. But phentolamine, an α antagonist, completely blocked NE- and ANG Ⅱ-induced stimulation of Na^+ uptake(phentolamine+NE+ANG Ⅱ : 25±3% inhibition vs. NE+ANG Ⅱ ; p < 0.05 ; Fig 9). In addition, atenolol(β_1 antagonist, 10^{-10}M) or butoxamine(β_2 antagonist, 10^{-10}M) was treated together with NE and ANG Ⅱ, NE- and ANG Ⅱ-induced stimulatory effect of Na^+ uptake was partially blocked. But propranolol(β antagonist, 10^{-10}M) completely blocked NE- and ANG Ⅱ-induced stimulation of Na^+ uptake(propranolol+NE+ANG Ⅱ : 24±6% inhibition vs. NE+ANG Ⅱ ; p < 0.05 ; Fig 11). These results suggest that α_1, α_2, β_1 and β_2 adrenoceptor are

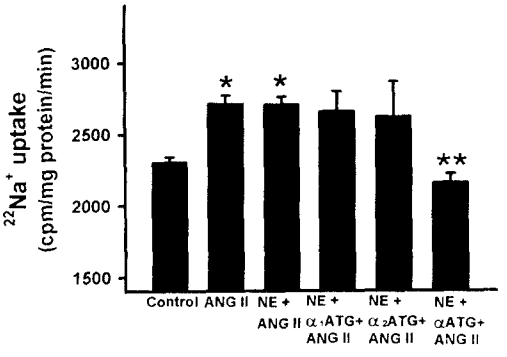

Fig 9. The interaction of between NE, α_1- or α_2-adrenoceptor antagonist(ATG) and ANG Ⅱ on Na^+ uptake. PTCs were incubated with ANG Ⅱ (10^{-11}M) alone or together with NE(10^{-9} M) for 4 hr under the treatment of vehicle, prazosin(α_1 ATG, 10^{-10}M), yohimbine(α_2 ATG, 10^{-10}M), or phentolamine(α ATG, 10^{-10}M) for 1 hr. Values are the means±S.E. of 9 experiments performed on 3 different cultures. *p < 0.05 vs. Control. **p < 0.05 vs. NE+ANG Ⅱ.

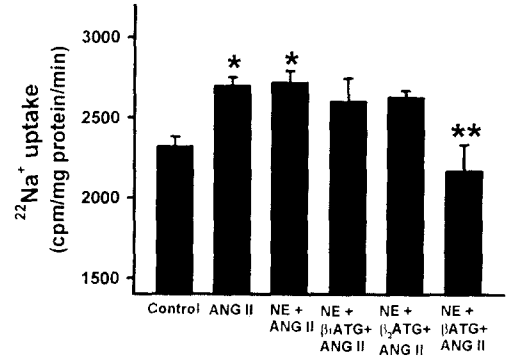

Fig 11. The interaction of between NE, β_1- or β_2- or β-adrenoceptor antagonists and ANG Ⅱ on Na^+ uptake. PTCs were incubated with ANG Ⅱ (10^{-11}M) alone or together with NE(10^{-9} M) for 4 hr under the treatment of vehicle, atenolol(β_1 ATG, 10^{-10}M), butoxamine(β_2 ATG, 10^{-10}M), propranolol(β ATG, 10^{-10} M) for 1 hr. Values are the means±S.E. of 9 experiments performed on 3 different cultures. *p < 0.05 vs. Control. **p < 0.05 vs. NE+ANG Ⅱ.

involved in the NE- and ANG Ⅱ-induced stimulation of Na^+ uptake.

Discussion

Renal sympathetic nerves participates in the regulation of the renal circulation, the filtration, reabsorptive, and secretory process involved in the renal handling of solutes and water[26]. An important neurotransmitter role for NE was supported by the observation that increasing renal sympathetic nerve activity by renal sympathetic nerve stimulation increased NE concentration in renal venous blood and/or urine [27]. In the present study, physiological concentration of NE increased Na^+ uptake. In the rat proximal tubule, NE caused a 30% increase in J_v and these effects were reversed by phenoxybenzamine, an α antagonist[28]. These results are consistent with our present study that phentolamine, an α antagonist, blocked NE-induced stimulation of Na^+ uptake, suggesting α receptors are involved in the NE-induced stimulation of Na^+ uptake. To better define the subtype of adrenoceptor on Na^+ transport, we conducted Na^+ uptake with the used of α_1 and α_2 agonist and antagonist. Only prazosin +yohimbine completely blocked NE-induced stimulation of Na^+ uptake. Furthermore, the α_1 agonist phenylephrine and the α_2 agonist clonidine stimulated Na^+ uptake. In isolated rabbit proximal tubule, α_2 adrenoceptors are predominant and involved in the stimulation of Na^+/H^+ exchanger[29,30]. Hesse and John also reported that, *in vivo*, in rabbit proximal tubule α_1-adrenoceptors influence renal nerve-stimulated Na^+ reabsorption[31]. In our present study, activation of either α_1 or α_2 receptors is associated with increased Na^+ uptake in the PTCs. In addition, β-adrenoceptors have been reported to be involved in the activation of fluid reabsorption in rabbit proximal tubule[32]. Although β_2 adrenergic receptor activation has been reported to be involved in increases in apical Na/H exchange as well as increases in transcellular Na^+ transport[33,34], the function of β_1 adrenoceptor has not been elucidated. However, present results suggest that as well as β_2, β_1 adrenoceptors also have an effect on Na^+ transport in the PTCs. All these results are partially inconsistant with researchers through at least two potential reasons such as difference in the experimental system(animal species ; *in vivo* vs. *in vitro* ; intact animal, organ or tissue vs. cultured cell) and various receptor calsses and subtypes defined by molecular cloning.

The signal pathways of respective subtype of adrenoceptors in the PTCs were not examined in the present study. In rabbit and rat renal tubule, β_1 and β_2 receptor subtype lead to the generation of cAMP by stimulating the enzyme adenylate cyclase[35,36]. However, isoprotenol-induced depolarization is independent of cAMP in rabbit proximal tubule[37] and β_2-adrenoceptor stimulate the Na^+ transport via the activation of PKC in rat proximal tubule[33]. Conversely, α_2-adrenergic receptor lead primarily to inhibition of adenylate cyclase. In constrast, α_1-adrenergic receptor activation leads to generation of the second messengers diacylglycerol and inositol triphosphate by stimulating the enzyme phospholipase C[38]. In rat proximal tubule, α_1-adrenergic agonist activate the chloride transport via PKC[39]. In our previous reports, phorbol esters stimulated the Na^+ uptake, while 8-Br-cAMP inhibited in the PTCs[18,40]. These results suggest that, in experimental model system, the stimulatory effect of adrenoceptors on Na^+ uptake primarily involved in the activation of PKC although we cannot rule out other pahtways.

The NE is thought to modulate blood pressure homeostasis through renal sodium flux in the proximal tubule as well as renin release and the renin-angiotensin-aldosterone system[41]. ANG Ⅱ increased Na^+ uptake via AT_1 receptor in the PTCs. These agents have not been investigated in terms of the interactions that they may have on proximal tubule Na^+/H^+ exchange although there are several reports in the kidney. In the opossum kidney cells β-adrenoceptors stimulate the expression of the angiotensinogen gene[42]. However in human kidney, functional β_2 adrenergic receptors are likely to be independent of local renin angiotensin system[43]. In our present studies there is no synergistic effect on stimulation of Na^+ uptake although ANG Ⅱ or NE alone induced stimulation of Na^+ uptake. Theses effects were also observed under the treatment of α_1, α_2, β_1 or β_2 receptor agonist. However, NE+α(or β) antagonist+ANG Ⅱ exhibited no stimulation of Na^+ uptake. These results suggest that there may be an interaction between adrenoceptor(α or β)

and ANG Ⅱ. Although the exact mechanism is not known, there are several evidences. In rabbit and human subjects, an α_1 adrenoceptor antagonist blunts the antinatriurectic effect of ANG Ⅱ[44,45] and angiotensin converting enzyme(enlapril) inhibition blunts the antinatriuretic effect of circulating NE[46].

In summary, in rabbit renal proximal tubule cells all of α_1, α_2, β_1, and β_2 receptor are involved in the NE-induced stimulation of Na^+ uptake. Furthermore α_1, α_2, β_1, and β_2 receptor may be involved in the interaction between ANG Ⅱ and NE on Na^+ uptake.

Conclusion

The present study was performed to determine the subtype of the adrenoceptors having an influence on Na^+ uptake and furthermore, to examine the interaction between NE and ANG Ⅱ on Na^+ uptake in PTCs. NE induced a stimulation of Na^+ uptake. α_1, α_2, β_1 and β_2 receptor agonist stimulated Na^+ uptake. $\alpha(\alpha_1+\alpha_2)$ or $\beta(\beta_1+\beta_2)$ receptor antagonist completely blocked NE-induced stimulation of Na^+ uptake. When NE and ANG Ⅱ were added together, there was no synergistic effect on Na^+ uptake. However, $\alpha(\alpha_1+\alpha_2)$ or $\beta(\beta_1+\beta_2)$ receptor antagonist blocked NE and ANG Ⅱ-induced stimulation of Na^+ uptake.

In conclusion, NE stimulates Na^+ uptake through α_1, α_2, β_1, and β_2 receptors. α_1, α_2, β_1, and β_2 receptors are also involved in the NE- and ANG Ⅱ-induced stimulation of Na^+ uptake.

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