DISCUSSION

These results demonstrated that an activator of endothelial NOS was present in the cortex of goat kidney, and in the absence of this activator the eNOS by itself showed very little enzymic activity for the production of NO. This protein activator was found to reduce the l-epinephrine induced elevated blood pressures (both systolic and diastolic) in rabbits.

Although this activator (cortexin), a protein of M, 43 kDa, was isolated from the cortex portion of goat kidney, the immunologic cross reactivity of the antibody raised against the purified protein indicated that cortexin was also present not only in rabbit, mice, men as described here, but tissue samples from guinea pig, cow and cat were also found to show cross reactivity against the cortexin antibody. These results might suggest that cortexin or cortexin like molecule from kidney could be of wide occurrence in the animal kingdom for the control of elevated blood pressure, and the occurrence of cortexin was not a goat kidney cortex specific phenomenon.

In the case of rabbit where the systolic and diastolic pressure were found to be 154 ± 4.02 mm of Hg and 59 ± 6.94 mm of Hg respectively under “normal” conditions, the injection of 136 nmol l-epinephrine / kg body weight increased both systolic and diastolic pressures within 30 min after administration of the catecholamine [Fig. 19]. The increased blood pressures were found to however continue to increase maximally to 208 ± 15.62 mm of Hg (systolic) and 98 ± 6.64 mm of Hg (diastolic) up to 3 h (n = 8). The elevated pressures remained roughly stationary at least for the next 45 h. The injection of 0.5 nmol of cortexin / kg body weight of the test animal decreased the systolic and diastolic pressures to 133 ± 12.14 mm of Hg (p<0.0005) and 51 ± 3.21 mm of Hg (p<0.0005) respectively within 30 min after the injection and was found to maintain the pressure at this “normal” level at least for the next 45 h when compared to the untreated control [Fig. 19]. The decrease of both diastolic and systolic blood pressures induced by the injection of cortexin was apparently mediated through the increase of NO level in the circulation due to the activation of endothelial eNOS. The injection of NAME, an inhibitor of NO synthesis [Sakuma et al, 1988] which impaired
the cortexin induced increase of NO in the system simultaneously negated the antihypertensive effect of the activator, indicating that the reduction of \( l \)-epinephrine induced high blood pressure by cortexin in rabbit was mediated through the systemic production of NO [Fig. 19], which has been reported to be a global vasodilator [Ignarro et al, 1986]. The vasodilatory effect of cortexin through NO synthesis in turn was found to mediate its antihypertensive effect via the formation of both cAMP and cGMP in the endothelial cells. Our results indicated that while the addition of 0.86 nM cortexin to the endothelial cell suspension increased the cellular cGMP level by 3.0 fold over the basal level, similar amount of the activator also increased cAMP level by 2.8 fold over the basal in these cells under identical conditions. Since the increased cellular level of either cAMP, or cGMP, has been reported to result in vasodilation [Karsten et al, 2003], the cortexin induced antihypertensive effect was related apparently to the increase of both the cyclic nucleotides to nearly equal extent in endothelial cells.

It was further found that the administration of polyclonal antibody raised against cortexin in normal rabbit which neutralized cortexin in the system resulted in the increase of both systolic and diastolic pressures in the animal with simultaneous reduction of the plasma NO level. It was found that not only the administration of cortexin “normalized” the elevated blood pressure in rabbit which was achieved through the injection of \( l \)-epinephrine but it was also found that the injection of the catecholamine resulted in the reduction of plasma NO level, and the treatment also led to the severe reduction of plasma cortexin level.

Although the mechanism of \( l \)-epinephrine induced inhibition of systemic NO production is not known, the inhibition of cortexin synthesis that was associated with the inhibition of NO production was not an isolated phenomenon. Systemic inhibition of NO production by NAME also resulted in the reduction of plasma cortexin level (0 pmol / ml). Conversely, \textit{in vitro} translation of cortexin synthesis in the cortex cell suspension indicated that NO itself was able to stimulate the synthesis of cortexin in these cells. In this context it could be suggested that even the antihypertensive effect of sodium nitroprusside (SNP), a NO generating agent \textit{in situ} [Lorenzo et al, 1971] might also be mediated through NO induced cortexin synthesis. Furthermore addition of NAME, an
inhibitor of NO synthesis, to the reaction mixture for in vitro translation of cortexin resulted in the complete inhibition of the synthesis of both NO and the hypotensive protein in the endothelial cells. The stimulation of cortexin synthesis by NO which in turn stimulated cortexin synthesis presented a “positive” feedback stimulation of both NO and cortexin. This behavior of NO as a positive feedback activator has been reported before [Bhattacharya et al, 2001].

The dose response increase of NO synthesis induced by cortexin in endothelial cells as well as the time course of the cortexin induced NO synthesis in endothelial cells [Fig. 16 and Fig. 17 respectively] together with the fact that eNOS by itself has little enzymic activity indicated that at least three different mechanisms were involved in the prevention of uncontrolled synthesis of NO catalyzed by eNOS itself and by the cortexin activated eNOS in the endothelial layer to forestall the development of hypotension due to overproduction of NO in the system. Although the exact mechanism for the regulation of the activated eNOS by cortexin is not known currently, these results suggested that the desensitization of the cortexin receptor binding sites at higher concentrations of the activator could be involved [Fig. 16]. On the other hand exhaustion of \( \alpha \)-arginine for the enzymic reaction in the time dependent inhibition of NO synthesis [Fig. 17] is also a possibility. However other mechanisms including the destruction of cortexin on the endothelial surface could not be excluded.

At present the mechanism of activation of eNOS by cortexin is not known. However our preliminary results indicated that the activation of eNOS by cortexin was mediated through the activation of tyrosine kinase in the endothelial membrane. However, it was also found that the NO induced vasodilation due to activation of eNOS by cortexin was also mediated through the activation of protein kinases in the endothelial cells.

As discussed above no definable cause for the essential (idiopathic) hypertension is yet known. However hypertension is itself a group of heterogeneous diseases and as such, variety of causes including renin dependence, salt sensitiveness, cell membrane defect, insulin resistance and many other factors have been reported to be involved in the development of essential hypertension in men [Messerli et al, 2007].
Furthermore it was also suggested that since the kidney is a rich source of rennin-angiotensin system and cortexin, also a kidney derived protein, it was possible that cortexin could be a component of the renal rennin-angiotensin system. However the role of cortexin in the system may be more complex than it was thought. Oral administration of captopril, a well-known angiotensin converting enzyme inhibitor (ACE inhibitor), to rabbit resulted in the reduction of normal plasma cortexin level from 229 ± 5.6 pmol / ml to 160 ± 2.5 pmol / ml (n=10, p<0.0005) in 2 h in these animals. These data however did not clarify the role of cortexin in the rennin-angiotensin system in the kidney as a whole.

Although whether the impaired production of cortexin had an indispensable role in the development of essential hypertension is not known, our results nevertheless suggested that this kidney derived protein might be one of the factors that could be involved in the genesis of this condition in men. It was noted that the plasma cortexin level in >98% of the newly diagnosed patients with essential hypertension, who had otherwise “normal” clinical profiles compared to normotensive volunteers [Table-1], was reduced below the detectable level as determined by the ELISA technique [Fig. 24].

As reported above, the coefficient of correlation (r) between the plasma cortexin level and blood pressures was within the acceptable ranges of -1≤ r ≤ +1. The coefficient of correlation “r” was always found to be negative irrespective of systolic or diastolic pressure either in the case of normotensive or in the case of hypertensive persons. The negative “r” values indicated that there was a negative correlation between these variables i.e. the variables changed in opposite direction implying that either systolic or diastolic pressure was inversely related to the plasma cortexin levels in both hypertensive and normotensive persons.

Although it was not possible for us to determine the effect of cortexin in subjects with established essential hypertension in men, the above results suggested that the systemic deficiency of the cortex derived factor could result in the development of essential hypertension in men at least indirectly.

The immunoblot analysis demonstrated that cortexin was present not only in the supernatant from the crude cortex homogenate from the goat kidney, but the activator
was also present in the circulation in men [Fig. 23]. The *in vitro* translation of cortexin synthesis in kidney cortex cells strongly suggested that this activator protein was synthesized in the cortex of kidney, and subsequently the antihypertensive protein appeared in the circulation for the maintenance of normotensive homeostasis. And as such, the activator protein satisfied the definition of a hormone [Gove et al (Editors), 1986] which was involved in the maintenance of normotensive conditions. Cortexin was found to be a stimulator of NO synthesis in the endothelial cells, and also in platelets. Cortexin was also found to be a potent inhibitor of platelet aggregation [Fig. 25]. The increase of platelet NO level is reported to inhibit platelet aggregation through the increase of both cAMP and cGMP level [Karsten et al, 2003; Kanowitz, 1981], in platelets. Numerous investigators reported before that although hypertension is a serious risk factor for the development of AMI and other coronary artery diseases, reduction of hypertension by currently available anti-hypertensive medication does not equally lead to the reduction of the coronary artery diseases [Messerli et al, 2007]. The inhibition of platelet aggregation indicated that the antihypertensive protein may prevent Acute Ischemic Heart Disease (AIHD, commonly known as heart attack which is the greatest killer of human race) in normotensive persons. These results may indicate that the control of hypertension by physiologic pathway is not equivalent to the control of hypertension by pharmacologic agents which may not stimulate the physiologic anti-hypertensive agent in the body. Here cortexin was found to be a physiologic agent for the control of hypertension through the increase of systemic NO production in endothelial cells.