نقش فاکتور شل‌کننده مشتق شده از آندوتلیوم (EDRF) در تنظیم تون عروقی

علی خوش‌باطن *

چکیده:

از زمانی که آمیل نیترات توسط توماس لودر- برانتون در کلینیک بعنوان یک داروی گشادکننده عروق معرفی شد، نیتراتها به صور مختلف و وسیع برای درمان آنژین صدری - انفارکتوس میوکارد و دیگر بیماریهای قلب و عروقی مورد استفاده قرار گرفت. در دهه گذشته توجه زیادی برای شناسایی مکانیسم اثر نیتراتها انجام گرفته‌است و این امر به دنبال کشف No آزاد شده از اندوتلیوم شدت بیشتری به خود گرفت.

در آزمایشات حاضر با استفاده از تکنیک پرفیوژن و لیزر داپلر فلومتری (LDF) اثرات بعضی از فعال کننده‌های عروقی (Vasoactive) و نقش سیستم عصبی کنترل کننده عروق در حضور و عدم حضور اندوتلیوم مورد بررسی قرار گرفته‌است. موادی مثل آدنوزین و آدنوزین تری فسفات (ATP) و استیل کولین (Ach) اثرات شل‌کنندگی خود را از طریق اندوتلیوم اعمال می‌کنند، و این اثرات وابسته به دوز نیز می‌باشند. اثر (Vasoconstriction) که بدنبال تحریک الکتریکی بوجود آمده بود در شرایطی که سنتز No بوسیله Nw-Nitro-L-arginine methyl-Ester(L.NAME) مهار شده بود نیز به میزان ۶۰ تا ۱۰۰ درصد افزایش یافته و این اثر با داروی دیگر مثل L-arginine که تولید No را افزایش می‌دهد حذف گردید.

این نتایج نشان می‌دهد که اندوتلیوم با آزادسازی No بطور طبیعی در تنظیم عروقی که تحت اعصاب سمپاتیک با اثر بعضی از مواد آندوژن موجود در خون می‌باشد نقش بسزایی دارد. حذف اندوتلیوم باعث افزایش بیشتر اثرات انقباضی مواد اندوژن و ایمپالسهای عصبی و در نتیجه اسپاسم‌های عروقی و ایسکمی‌می‌گردد.

کلید واژه‌ها: ۱ـ EDRF ۲ـ تونوس عروقی ۳ـ نیترووازودیلاتورها

* بخش فیزیولوژی و فیزیک پزشکی دانشگاه علوم پزشکی امام حسین (ع) تهران

THE ROLE OF ENDOTHELIUM DERIVED RELAXATION FACTOR (EDRF) IN VASCULAR TONE CONTROL

Ali Khoshbaten *

ABSTRACT

Ever since Thomas Lauder - Bruton First introduced Amylnitrate into clinical medicine, nitrovasodilators of different classes have been extensively used in the medical treatment of angina pectoris, myocardial infarction and heart failure. Understanding the mechanisms of action of nitrates has been greatly expanded in the last decade, particularly since the discovery of endothelium dependent relaxation and its mediator, the endogenous nitrate, NO.

In present research, by using perfusion, isolated vessels´ring (in-vitro) and laser Doppler flowmetry, (LDF) in-vivo techniques, the effect of some vasoactive substances and vascular nervous system were studied in present and absent of endothelium. Substances such as Adenosine, Adenosine triphosphate (ATP) and Acetylcholine (Ach) induced their vasodilation effect via endothelium. These responses were dose dependent .

The vasoconstriction effect which followed, after nervous stimulation or vasoactive substances such as adrenergic agonists (clonidine, adrenaline and epinephrine) were augmented 60-100 percent, when NO. Synthesis was blocked by Nw-Nitro-1-arginine methyl-ester (L-NAME). This response was reversed by L-Arginine which induced NO synthesis.

These results showed that ednothelium has role in modulation of vasoactivity of blood vessels due to stimulation of sympathetic nerves or endogenous substance in blood by releasing NO.

Key Words: *1) EDRF* *2) Vascular Tone* *3) Adrenergic agonists* *4) Nitrovasodilators*

* Dep. of Physiology and Biophysics, College of Medicine, Baghiatallah (A.T) of Medical Sciences

INTRODUCTION

It is known that resistance of blood flow is the target of numerous control mechanisms in the peripheral circulation. Vascular resistance is determined by factors affecting either vascular (diameter, length, etc) or rheological (viscosity, haematocrit) parameters. The interaction among these factors to provide the actual resistance, however is not clearly and completely understood. Beside central factors there are local factors and changes of vascular which regulate micro-circulation to tissues. Among them the endothelium of blood vessel play a key role in the local control of blood vessels function.

The vascular encothelium is a monolayer of squamous cells covering the inner surface of all blood vessels. Although the endothelium constitutes only a thin layer of cells, but its over all volume in entire cardiovascular system is as large as the liver [7].

So far, many functions were stated for vascular endothelium in circulation, e.g., production of factors for blood clotting (like, von willebrand factor VIII, plasminogen activators and inhibitors), capillary transport and exchanges mechanism between blood and tissue. Activation and inactivation of some circulating hormones (norepinephrine serotonin, bradykinin and ADP), and the most recent finding production and release of vasoactive substances such as prostacyclin, endothelium derived relaxing/constrictor factors, prostanoids, angiotensin and histamine.

Many substances produced and/or released from the endothelial layer can affect the vascular smooth muscle tone (Fig.1): (1) prostacyclin and other prostanoids [15,17]; (2) angiotensin II either produced locally or taken up from the circulation [2,3]; (3) endothelium - derived relaxing factor (s) [6]

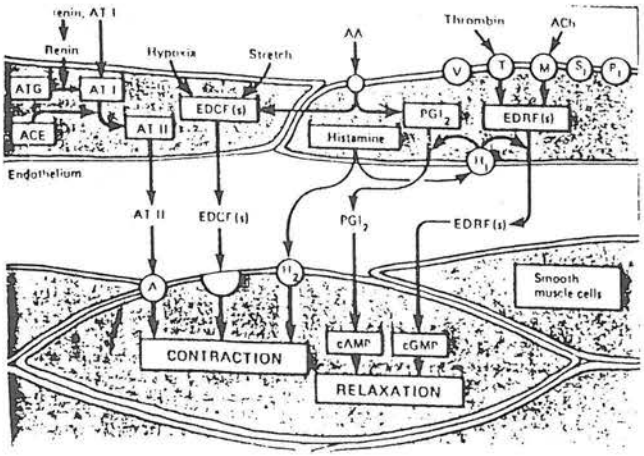

Fig.1 vasoactive substances released from the vascular endothelium. AA= Archidonic acid; ACE = angiotensin-converting enzyme; ACh = acetylcholine: ATI/II = angiotensin I/II; cAMP = cyclic adenosine monophosphate; ATG = angiotensinogen; cGMP = cyclic guanosine monophosphate; EDCF(s) = endothelium -derived constrictor factor (s); EDRF (s) = endothelium derived relaxing factor (s); 5-HT = 5-hydroxytryptamine (serotonin); PGI_2= prostacyclin. Receptors are represented by an open circle; A = angiotensinergic; H_1/H_2 = histaminergic receptor; M = muscarinic receptor; P_1 = purinergic receptor; S_1= serotonergic vasopressinergic receptor.

and endothelium-derived constrictor factor (s) under certain conditions such as hypoxia and stretch [13,19].

The objectives of the study are to find out the role of endothelium in regulation of blood flow by nervous and endogenous substances such as ACh, Purines, Adrenaline and etc in different vascular beds.

Different Methods were used in this study

A.In vitro experiments rabbits

White New Zealand rabbits weighing ~ 3kg or guinea pigs 400-500 grams were anaesthetized by a combination of an i.p., injection of diazepam (o.s mg/kg) and an i.m injection of fentanyl/fluanisone mixture (hypnorm, 0.2 ml/kg; janssen) and sodium pentobarbital (45 mg/kg i.p) respctively. Tissues preparations were made according to techniques described previously [5,9,12]

Removal of endothelial layer in these preparations were achieved by either perfusion of sodium deoxycholic acid 91-3mg/ml) for about two minutes or mechanical rubbing or adding N_w - Nitro - L-Arginin (NLA) into the organ both.

B. In vivo experiments

Experiments were carried out in New zealand rabbits of either sex weighing approximately 1.8 to 3kg. They were anaesthetized initially with hypnorm (0.1 mg/kg, i.m) and diazepam 0.5 mg/kg i.p) and this anaesthesia was maintained there after by using a halothane (1-2%), N_2 O/o_2

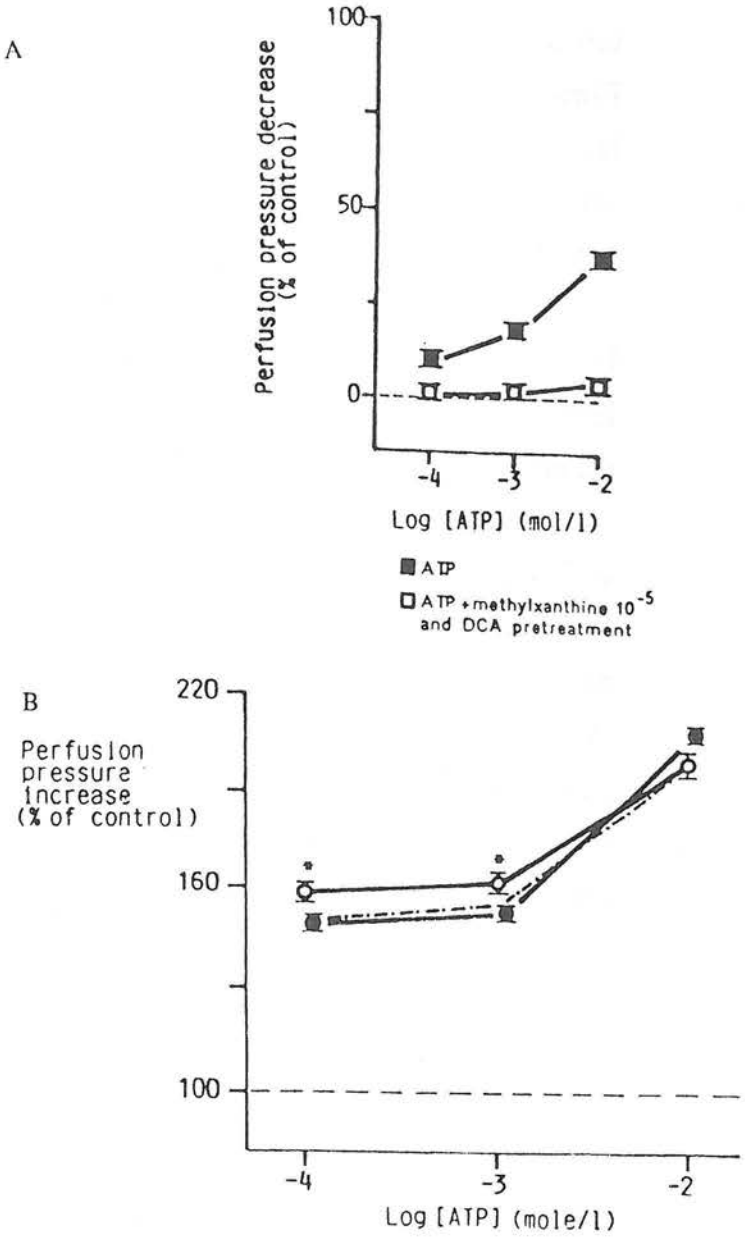

Fig.2: A. Dilator response to injected ATP before and after removal of the endothelial layer by DCA pretreatment. Vascular tone was elevated by perfusion with 5HT (10^{-5}M). n= 6-11.B. The constrictor response to injected ATP before and after removal of the endothelial layer by DCA pretreatment . The dot-dash line shows the response to ATP during perfusion with methylxanthine (10^{-5}M). In both cases vascular tone was elevated by perfusion with 5HT (10^{-5}M). n-6-7: P<0.05.*

which was delivered via a tracheal cannula. Further details of surgical procedures blood flow monitoring, drug administration and stimulus parameters were explained by Khoshbaten and Ferrell [5,9,10,11].

Removal of endothelium was achieved by infusion of L-NAME 3mg/kg via arterial line.

C.Statistics

Statistical data analysis was carried out by either paired or unpaired student t test (one-tailed). An F test was used to test the assumption of homogeneity of variances. Where this exceed tabled f values, the modified t values were generated using the formula described by Phillips [18]. All data expressed in the text and on the graphs are means ± SEM. Differences between means were considered significant of the p values were <0.05.

RESULTS

The effect of adenosine triphosphate (ATP) and acetylcholine

ATP produce a dual effect consisting of a transient constriction followed a long lasting dilatation. The removal of endothelium by deoxycholic acid not only increasd the constrictor response of ATP but abolished its dilator response too (Fig.2 a,b). Acetylcholine injection produced pronounced vasodilation which this response was also blocked by removal of endothelial layer (Fig.3).

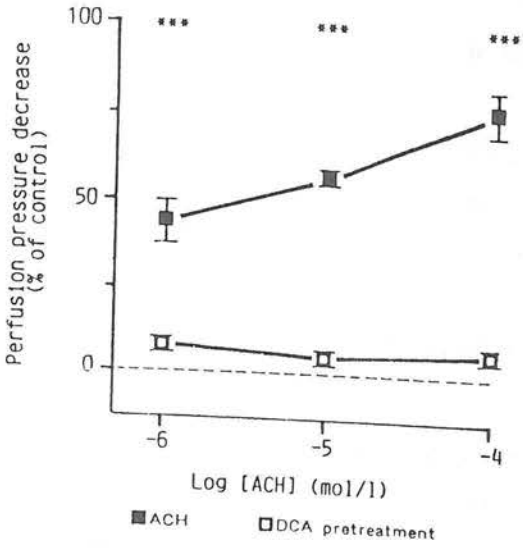

*Fig 3: The dilator effect of acetylcholine (n= 9-12) before and after removal of the endothelial layer by DCA pretreatment (n= 6-8). Vascular tone was raised by vasopressin (10^{-8}M). ***: P<0.001.*

Electrical stimulation of saphenous nerve

Electrical stimulation of saphenous nerve induced a repeatable reduction in articular blood flow during the period of stimulation, indicating vasoconstriction of knee joint blood vessels. Following infusion of L-NAME (3 mg/kghr) via saphenous artery near the knee joint, augmented the reconstirctor response due to nerve stimulation by 60 to 100% (Fig. 4). These responses returned to control values after infusion of L-arginine (Fig.4).

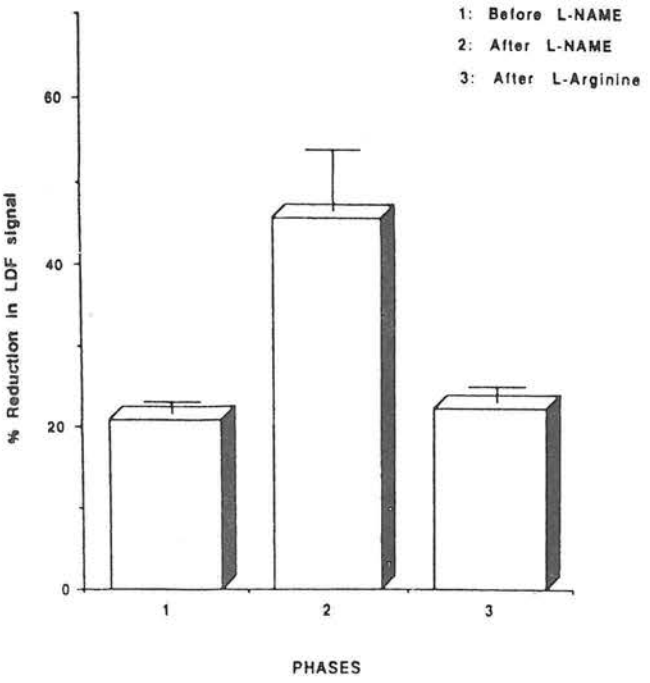

Fig.4: The vasoconstrictor response of articular blood vessels to verve stimulation (1OV, 1OHz, 1msec) under control concitions (phase 1) is enhanced during L-NAME perfusion (phase 2) and returned to control during L-arginine perfusion (phase 3). Means ± SEM, n= 5-6.

The effect of α agonists

In rabbit constrictor responses to close intra-arterial injection of phenylephrine (α_1 agonist) clonidine and UK 14304 (α_2 agonists) in vivo were also increased during infusion of L-NAME, although the magnitude of these changes varied between these agents (Fig.5).

In Vitro study on guinea pig thoracic aorta did also indicate the increase in vasoconstrictor responses of phenylephrine and clonidine after removal of endothelium mechanically or by Nw-Nitro-L-arginin (NLA). (Fig. 6a,b)

The dilatory effect of clonidine was also attenuated after NLA and removal of endothelium (Fig.6c)

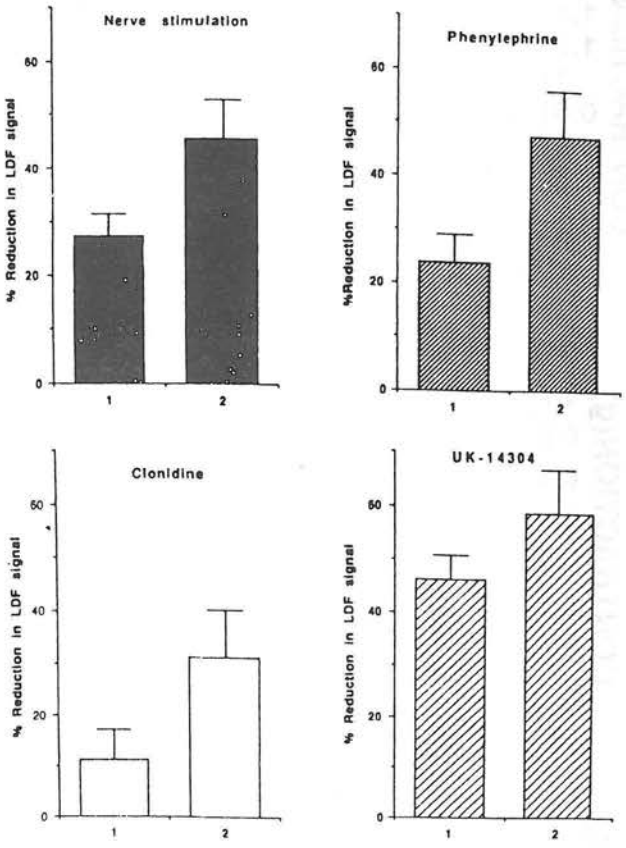

Fig.5: Vasoconstrictor response of articular blood vessels to nerve stimulation (lOV, lOHz, 1 msec), phenylephrine, clonidine, and UK14304 under control conditions (1) and during infusion of L-NAME (2). Mean ± SEM, n=5-6.

DISCUSSION

The prupose of the present study was twofold. Firstly, to establish the existence of receptors such as purinoceptors, adrenoseptors, and cholinergic receptors on vascular endothelium in appropriate vessels. Secondly, to investigate the modulatory effect of endothelium in these blood vessels.

The results of the present experiments demonstrate the dual effect of ATP on

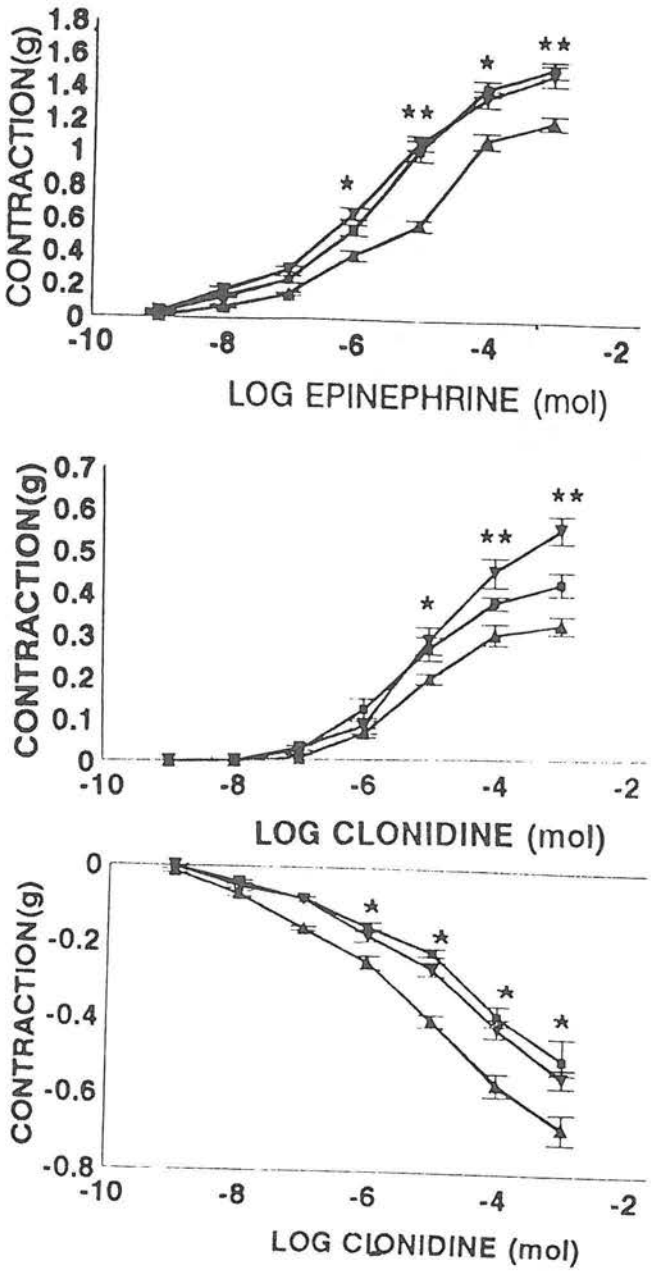

Fig.6: The effect of ephinephrine, clonidine on thoracic aorta of guinea pig with and without endothelium (: Mechanical removal, NLA pretreated). n=6. P<0.05**. P<0.005.*

articular blood vessels in the rabbit, ATP can produce vasoconstriction and vasodilation which after removal of endothelium, the former was augmented and the latter was abolished.

Acetylcholine was also found to exert a dilator effect which was consistently observed after increasing the vascular tone. This was, however virtually abolished by removal of the endothelium, which is similar to the results obtained by Furchangott and Zawadski (198)) on rabbit thoracic aorta.

Enhancement of vasoconstrictor effect of epinephrine and clonidine in guinea pig thoracic aorta after using NLA (blocker of No synthesis, EDRF) or mechanical removal of endothelium were similar to the results of other investigators [1,15,16].

In other in vivo experimets, the results demonstrate that as in other vascular beds, articular blood vessels in the rabbit knee are innervated by sympathetic efferent fibers. Electrical stimulation of the saphenous nerve induce vasoconstriction in these vessels. L-NAME augmented the responses to nerve stimulation and injection of phenylephrine an α_1 agonist, clonidine and UK14304 α_2 agonists. These were consistent with other studies as mentioned. The physiological significance of different distribution of receptors in different vascular beds were not completely understood. However these results suggest that endothelium by releasing substances like EDRF may play as important regulatory role in moderating the vasoconstrictor effects of sympathetic nerves innervating blood vessels and endogenous substances circulating in blood.

REFERENCES

1) Carrier GO, (1985). White RE: Enhancement of alpha$_1$- and alpha$_2$ - adrenergic agonist-induced vasoconstriction by removal of endothelium in rat aorta. J Pharm Exp ther; 232: 682-687.

2) Dzau VJ, (1984). Vascular wall renin angiotensin pathway in control of the circulation. Am J Med; 77:31-36.

3) Dzau VJ, (1986). Significance of the vascular renin-angiotensin pathway. Hypertension; 8: 553-559.

4) Ferrell, W.R. and Khoshbaten, A. (1989). Adrenoceptor profile of blood vessels in knee joint of the rabbit. J. of Physiology; 414: 377-383.

5) Ferrell, W.R. and Khoshbaten, A. (1990). The role of the endothelium in mediating the action of ATP, adenosine, and acetylcholine on flow through blood vessels in the rabbit knee joint. British J. of Pharmacology ; 99: 379-383.

6) Furchgott RF, Zawadzki JV, (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine, Nature; 299:373-376.

7) Hütter I, Gabbiani g, (1983). Vascular endothelium in hypertension. In: Hypertension, J Genest, Okuchel, P Hamet, M Cantin eds, Mc Graw-Hill, New York; PP: 473-488.

8) KHOSHBATEN, A. AND FERRELL, W.R. (1990). Response of blood vessels in the rabbit knee to acute joint inflammation. Journal of Annal Rheumatoid Disease; 49:540-544.

9) Khoshbaten, A. and Ferrell, W.R. (1990). Responses of rabbit articular blood vessels to nerve stimulation. Blood Vessels; 27, 41p.

10) Khoshbaten, A. and Ferrell, W.R. (1993). Nerve mediated responses of blood vessels in the rabbit knee joint . Journal of Vascular Research. 30,(2): 102-107.

11) Khoshbaten, A. W.R. Ferrell, and H. Najafipour. (1992). Modulation of nerve mediated responses in rabbit articular blood vessels by endothelium drived relaxing factor. Journal of Vascular research.

12) Khoshbaten, A. Norozian, Sharifi, A.M. (1995). Involvement of α_1 and α_2 adernoceptors in modulating vascular tone via endothelium in Guinea-pig thorasic aorta. European Journal of Physiology. supplement to Volume 430, No. 4,p 139.

13) Katusic ZS. Shepherd JT, Vanhoutte PM, (1987). Endothelium dependent contrations to stretch in canine basilar arteries. Am J. Physiol; 252:4671-673.

14) Lüscher TF, Romero JC, Vanhoutte PM, (1986). Bioassay of endothelium derived vasoactive substances in the aorta of normotensive and spontaneously hypertensive rats. J.Hypertension; 4 (suppl. 6): 81-83.

15) Lücher TF, Katusic ZS, Weber E, Bühler FR, (1987). Endothelium-dependent

relaxation are impaired in the renal carotid artery of spontaneously hypertensive rats (Abstract). Hypertension; 10:355.

16) Martin W, Furchgott RF, villani GM, J Othianandan D, (1986). Depression of contractile responses in rat aorta by spontaneously released endothelium derived relaxing factor. J Pharm Exp ther; 237: 529-331.

17) Moncada S, Vane JR, (1979). Pharmacology and endogeneous roles of prostaglandin endoperoxides, thromboxane A_2 and prostacyclin. Pharmacol Rev; 30: 293-331.

18) Phillips Ds, (1978). Basic statistics for health science students. San Francisco, Freeman.

19) Rubani GM, Vanhoutte PM, (1985). Hypoxia releases a vasoconstrictor substance from the canin vascular endothelium. J Physiol (Lond); 364: 45-56.