Re-evaluation of the Effects of NSAIDS on Blood Vessels

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Background: Blood vessels regulate peripheral resistance and hence blood pressure. Their tone is controlled by autonomic nervous system, calcium influx and regulation of cycloxygenase-2 (COX-2). Autonomic nervous system also regulates calcium influx in to the cells. Some of NSAIDs like salicylates block calcium influx across the cell membrane. We have tried to find out effects of diclofenac, piroxicam and celecoxib on isolated blood vessel and their role in hypertension.

Methods: We have found effects of diclofenac, piroxicam and celecoxib on the rat aortic ring with intact endothelium and spiral strip of rabbit portal vein. The isolated tissues were stimulated with 1 μM of norepinephrine. Diclofenac 15 and 20μM and piroxicam 15 μM produced significant relaxation in rat aortic ring while there was no significant effect of celecoxib. Diclofenac 10, 15 and 20 μM, piroxicam 5 μM, celecoxib 5, 10 and 20 μM produced significant vasorelaxant effects on rabbit portal vein.

Conclusions: It is concluded that only non specific COX inhibitors affect arteriolar tone while venous tone is also decreased significantly not only by nonspecific but also by COX-2 inhibitors.

Key Words: Blood vessels, NSAIDs, Endothelium, Hypertension

Introduction

While investigating the membrane properties of vascular smooth muscle, regional differences become evident. For example, spontaneous membrane activity and spontaneous contractions are recordable only from the smooth muscle of the portal vein.[1] The movement of calcium though calcium channels is controlled by electrical potential, they have been termed as potential or voltage-operated channels. Another group of calcium channels is sensitive to control by membrane receptors. It has been established that β_1 adrenergic agonists in cardiac muscle and α- adrenergic agonists in vascular smooth muscle increase calcium influx via the slow-inward current. Thus they enhance contractility, frequency and conduction velocity in the heart in case of β_1- receptors, and the degree of contraction of blood vessels in case of α-receptors. These receptors or agonist-mediated channels are termed as receptor-operated channel.[2,3] Contraction or force development by smooth muscle cells depends upon the elevation of intracellular calcium in the myoplasm. This is caused by three mechanism (i) release of intracellular calcium from the storage sites like mitochondria, and cisternae (ii) entry of calcium via voltage –operated calcium channels and (iii) entry of calcium via receptor-operated channels. [2] It has been demonstrated that potassium chloride induced contractions of the rabbit aorta depend almost entirely upon the presence of extracellular calcium whereas both extracellular and intracellular calcium pools are utilized during noradrenaline-induced contractions of the tissue.[4] In the portal vein all responses appear to depend exclusively upon extracellular calcium. However the relative importance of different sources of calcium for contractions varies considerably in blood vessels from different anatomical positions even under similar experimental conditions.[5] In general, the contribution of cell-derived calcium is greater in arteries than in veins.[6]

Materials and Methods

Animals: Albino rats (100-300 G) of either sex were received from the National Institute of Health, Islamabad. They were kept in the animal house of Department of Pharmacology, King Edward Medical University, Lahore, and provided with liberal amount of water and food. The sipper water bottles were washed daily and filled with fresh clean water. Male rabbits (1-2 kg) were purchased from the local market of Lahore and Veterinary Research Institute, Lahore. They were kept in a separate animal house and were provided with liberal amount of water and food. Initially all groups were fed on normal diet for a period of two weeks for acclimatization before starting the experiment.

Chemicals: The chemicals were used in the following compositions to make the Krebs solution in G/L (Sodium Chloride 6.9, Potassium Chloride 0.35, Calcium Chloride 0.28) Magnesium Chloride 0.29, Potassium Dihydrogen Phosphate 0.16, Sodium Bicarbonate 2.1, Glucose 2.1 [7,8] and Ringer's Lock Solution in G/L (Sodium Chloride 1.9, Potassium Chloride 0.42, Calcium Chloride 0.24, Sodium Bicarbonate 0.15, Glucose 1.0).

Drugs: Diclofenac sodium 50 Gms and piroxicam 50 Gms were obtained on request from Siza International, Pakistan.

Celecoxib 100 Gms and Polyethyleneglycol (solvent) 50 Gms was obtained from Highnoon Laboratories, Pakistan. Norepinephrine 4mg/ml Vials Bedford laboratories, Bedford were also used in the research.

The Krebs solution was freshly prepared daily. The chemicals were weighed out. Sodium chloride, glucose and sodium bicarbonate were weighed out daily for preparing the Krebs solution, whereas stock solutions were made of the rest of the ingredients. It is best to add calcium salt last, after all the other salts have been thoroughly dissolved in distilled or deionized water. Otherwise the poorly soluble calcium salt may precipitate. The solution in the organ baths was kept at 37°C, the temperature being controlled with a thermostat fixed inside the chamber. Krebs solution was continuously aerated with a mixture of 5% carbon dioxide and 95% oxygen.

Surgical Instruments: Scissors, iris Scissors, iris dissection forceps, arterior forceps, curved forceps, straight forceps.

Equipments: UPS, weighing balance (for small animals).A power lab 4/25 (AD Instrument Australia) were used in our experiments. Isotonic transducer (0-25G) MLT0015/D and teaching force transducer (0-25G) MLT 0210 /A were used for spontaneous activity of isolated blood vessels. Automatic Organ Baths, Letica were used for the study.

Grouping of animals: Grouping of the animals study was done as follows:

Group I (n = 18)
In this group rat aortas were used. This group was divided into three subgroups.

I D (n = 6) This group was treated with diclofenac (10µM, 15µM, 20µM).
I P (n = 6) This group was treated with piroxicam (5µM, 10µM, 15µM).
I C (n = 6) This group was treated with celecoxib (5µM, 10µM, 15µM).

Group II (n=18)
In this group rabbit portal veins were used. This group was divided into three subgroups.

II D (n = 6) This group was treated with diclofenac (10µM, 15µM, 20µM).
II P (n = 6) This group was treated with piroxicam (5µM, 10µM, 15µM).
II C (n = 6) This group was treated with celecoxib (5µM, 10µM, 15µM).

Rat Aortic Rings

The rat was stunned, its cervical dislocation was done and chest was opened. The lungs and heart were pushed aside so as to have a clear view of the thoaracic aorta. The aorta descends down, after arching, along the spinal column and enters the abdomen after going through the diaphragm. It was obtained with a pair of forceps and was cleaned of connective tissues and fat in situ rapidly. One cut was given as near the heart as possible and another one 2-3 cm down the first one. The animal was then transferred to a petri dish containing aerated Krebs physiological salt solution. The aortas were further cleared of connective tissue with the help of a pair of iris scissors and forceps. Four to five aortic rings were cut. The tissue was kept moist with Krebs solution during the procedure. The aortic ring was passed through u-shaped coils made with the help of common pins. These pins were attached to thread and mounted to forced transducer and lever in organ bath. Care was taken as it should not be crushed with the instruments. The solution in the organ bath was constantly aerated with small bubbles of a mixture of 95% oxygen and 5% carbon dioxide, and kept at 37°C with the help of a thermostat. The transducer was coupled to and calibrated with the physiograph for recording the isometric tension of the tissue. A resting tension of 2-4 G was applied to the tissue throughout the experiment. [5,9] The preparation was allowed to settle and equilibrate with the solution for 30-45 minutes. During this time, the solution was changed at intervals of 15-20 minutes. [9]

Rabbit Portal Vein

Rabbit was sacrificed by cutting its throat. The abdomen was opened and its contents exposed. The stomach and intestines along with the mesentery was pushed to the left side of the animal. The portal vein was seen over the surface of the mesentery going up to the liver. It was distinguished from the bile duct by its blue colour, the bile duct was more firm and whitish, and entered into the duodenum. The vein was obtained with the help of iris forceps and cleared of connective tissue and fat in situ. A 20-30 mm long segment was separated from it and immediately transferred to a dish is aerated with 5% CO_2 and 95% O_2 mixture. The vein was mounted on glass rod and spiral stripe was made. The tissue was mounted in the chamber and attached to the polygraph. The segment was set up under a resting tension of 0.5-1G throughout the experiment.[10] The portal vein was allowed to equilibrate for a period of 20 minutes to 30 minutes.[8] The Krebs solution was changed every 10 – 15 minutes during the equilibration period. The contractions were induced with 1µm noradrenaline. The tissues contracted. The contraction was recorded. The tissue was washed for 2-3 minutes and allowed to relax for thirty minutes.

Mechanical Recording of Rat Aortic Ring and Rabbit Portal Vein

The aortic strip was mounted in the double organ baths under resting tensions of 2-4 G. After the usual equilibration time contractions were induced with 1µM noradrenaline. I µM noradrenaline was the final calculated dose in the Krebs solution. Noradrenaline was added to the baths with the help of pipettes or an insulin syringe. Care was taken that it should not be splashed over the tissues. The tissue immedi-

ately contracted. The contractions were recorded. The contractions of the tissues (isometric tension) were recorded for 30 minutes. After recording the response of the tissues to the agonist, the recording was stopped. The tissues were washed 2-3 times and allowed to relax for 30 minutes. The tissues were again induced to contract with 1μM noradrenaline, the recordings allowed for 30 minutes again. This process of induction of contractions was repeated till we got two almost equal responses not varying more than 10% of each other in amplitude. The last response was considered as control. It was quantified by measuring the force (weight) of contractile response. Since significant release of intracellular calcium in vascular muscle only occurs following stimulation with concentrations of the agonist (noradrenaline). The concentration of noradrenaline used in this study (I μM) produced contractions which were approximately 90% of maximum. Moreover, all responses investigated in the present study could be elicited at 30 minutes intervals over a period of hours with less than 10% variation.[5] After recording the control responses with noradrenaline for five minutes, the tissue was treated at the peak level of contraction at interval of 5 minutes, with three increasing concentrations of NSAIDs.

Statistical Analysis

The use of statistical principles was made for conducting our research experiments. For this purpose, animals were randomly selected from the same progeny and groups of 6 animals for each group were formed. The data were collected on pharmacological parameters and statistically analyzed to test various null hypotheses about the mean values of these parameters. Treatments were estimated at 5% level of significance. Paired 't' test was performed.

Results

1. **Effects on Rat Aorta**

   a. ***Effects of Diclofenac***

   Diclofenac 15μM and 20μM produced significant relaxation in the norepinephrine induced contraction in the rat aortic ring with intact endothelium. The effect produced with 10μM was also relaxant (Table 1 and 3).

   b. Effects of Piroxicam

   Piroxicam, 5, 10 and 15μM produced relaxation in the rat aortic ring. However the effect produced with 15μM piroxicam was significant (Table 1 and 3).

   c. Effects of Celecoxib

   Celecoxib 5, 10 and 15μM produced nonsignificant vasorelaxant effects (Table1 and 3).

Table 1: *Response [Contractility (Height In Grams)] of The Rat Aorta to Norepinephrine in the Absence (Control) and Presence of Different Concentrations of NSAIDs.*

Mean of six experiments	**Diclofenac 10 μM**		**Diclofenac 15 μM**		**Diclofenac 20 μM**	
Norepinephrine (1μM)	**Control**	**Test**	**Control**	**Test**	**Control**	**Test**
Mean	2.653	2.497	2.497	2.187	2.187	1.897
+S.E	0.2672	0.2876	0.2876	0.3241	0.3241	0.3442
"p" value	0.6399		0.0218		0.0389	
	Piroxicam 5 μM		**Piroxicam 10 μM**		**Piroxicam 15 μM**	
Mean	2.855	2.837	2.837	2.448	2.448	2.383
+S.E	0.3061	0.3137	0.3137	0.2789	0.2789	0.2998
"p" value	0.3588		0.3316		0.0572	
	Celecoxib 5 μM		**Celecoxib 10 μM**		**Celecoxib 15 μM**	
Mean	2.44	2.37	2.37	2.238	2.238	2.015
+S.E	0.2821	0.3169	0.3169	0.364	0.364	0.334
"p" value	0.6961		0.1914		0.1093	

Table 2: *Response [Contractility (Height in Grams)] of The Rabbit Portal Vein to Norepinephrine in the absence (Control) and Presence of Different Concentrations of NSAIDs.*

Mean of six experiments	**Diclofenac 10 µM**		**Diclofenac 15 µM**		**Diclofenac 20 µM**	
Norepinephrine (1µM)	**Control**	**Test**	**Control**	**Test**	**Control**	**Test**
Mean	2.872	2.398	2.872	2.012	2.872	1.657
+S.E	0.2692	0.277	0.2692	0.2833	0.2692	0.3848
"p" value	0.0071		0.0101		0.0255	
	Piroxicam 5 µM		**Piroxicam 10 µM**		**Piroxicam 15 µM**	
Mean	2.639	2.374	2.639	2.604	2.639	2.349
+S.E	0.5448	0.4968	0.5448	0.6006	0.5448	0.5572
"p" value	0.0046		0.9425		0.4865	
	Celecoxib 5 µM		**Celecoxib 10 µM**		**Celecoxib 15 µM**	
Mean	2.51	2.123	2.51	1.828	2.51	1.493
+S.E	0.5473	0.4533	0.5473	0.4678	0.5473	0.5407
"p" value	0.0268		0.0052		0.0062	

Table 3: *%Changes Observed In Response[Contractility (Height In Grams)] Of The Rat Aorta To Norepinephrine In The Absence(Control) And Presence Of Different Concentrations Of NSAIDs*

Mean of six experiments	**Diclofenac 15 µM**			**Diclofenac 20 µM**			**Piroxicam 15 µM**		
Norepinephrine (1µM)	**Control**	**Test**	**% Contraction**	**Control**	**Test**	**% Contraction**	**Control**	**Test**	**% Contraction**
	3.55	3.39	95.49295775	3.39	3.16	93.21533923	3.43	3.43	100
	2.71	2.07	76.38376384	2.07	1.44	69.56521739	1.75	1.63	93.14285714
	2.47	2.28	92.30769231	2.28	1.88	82.45614035	2.63	2.62	99.61977186
	2.02	1.97	97.52475248	1.97	2.12	107.6142132	2.44	2.3	94.26229508
	2.75	2.47	89.81818182	2.47	2.16	87.44939271	2.83	2.82	99.64664311
	1.48	0.94	63.51351351	0.94	0.62	65.95744681	1.61	1.5	93.16770186

2. **Effects on Rabbit Portal Vein**

   a. **Effects of Diclofenac**

   Diclofenac 10, 15 and 20µM produced statistically significant vasorelaxant effect on norepinephrine induced contraction of rabbit portal vein (Table 2 Figure 1)

   b. **Effects of Piroxicam**

   Piroxicam 5µM produced highly significant vasorelaxant effect on rabbit portal vein. However effects produced with 10 and 15µM piroxicam were also vasorelaxant (Table 2 Figure 2).

   d. Effects of Celecoxib

   Celecoxib 5, 10 and 15 µM produced highly significant vasorelaxant effect on rabbit portal vein. (Table 2 Figure 3).

Discussion

The smooth muscle cells contract whenever the intracellular calcium level increases. Smooth muscle cells of the vessel

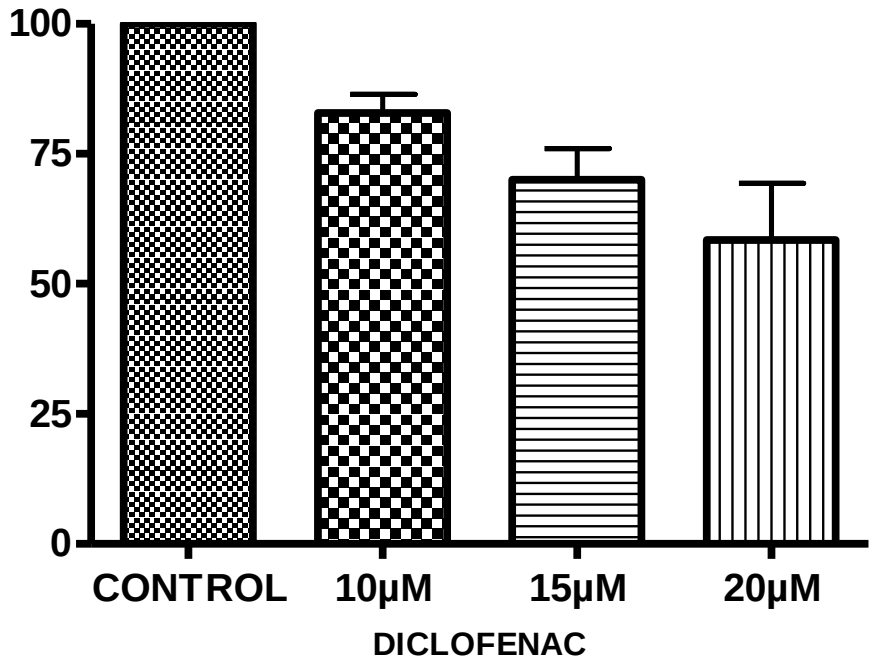

Fig 1. %CONTRACTION OF RABBIT PORTAL VEIN CAUSED BY NOREPINEPHINE(1µM) ALONE (CONTROL)AND IN THE PRESENCE OF DICLOFENAC

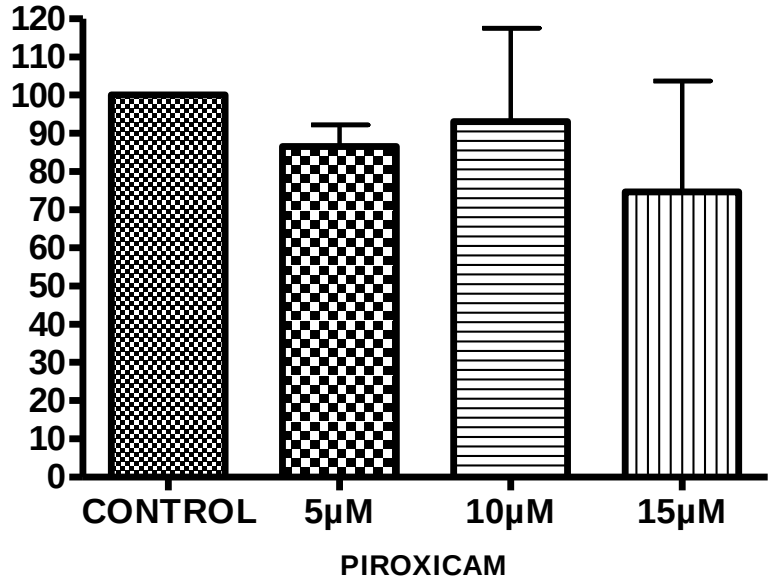

Fig 2. %CONTRACTION OF RABBIT PORTAL VEIN CAUSED BY NOREPINEPHINE(1µM) ALONE (CONTROL) AND IN THE PRESENCE OF PIROXICAM

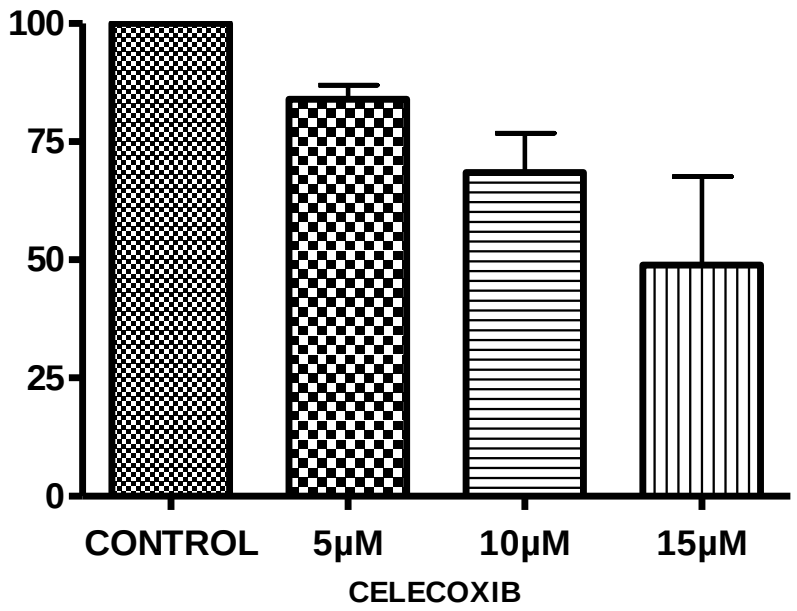

Fig 3. %CONTRACTION OF RABBIT PORTAL VEIN CAUSED BY NOREPINEPHINE(1µM)ALONE(CONTROL)AND IN THE PRESENCE OF CELECOXIB

wall thus control the size of the luminae of vessels regulating the circulation and distribution of blood in various parts of body. The muscle tone of peripheral vascular tissues contributes to the regulation of blood pressure and is controlled by myogenic, neurogenic and humoral factors.[11] The properties of smooth muscle cells in vascular tissue differ with the region and species. Therefore, to make a strict comparison of drug action in a vascular bed in vitro, the same species and the same region should be used.[12] The differences in sensitivity of smooth muscle cells in various blood vessels depend upon the types of receptors in their plasma membrane. The adrenoceptor system in vascular smooth muscle and in particular responses mediated by alpha-adrenoceptor subtypes have been a major focus of attention for the action of various vasodilators and in particular the calcium channel blockers, because of their contribution to vascular tone.[9] Vessels (like saphenous vein) which contain α1 and α2 – adrenoceptors behave differently than the portal vein which contains α1 adrenoceptors exclusively when both the veins are acted upon by the same vasodilator. Similarly the response of thoracic aorta of rabbit to a vasodilator is different from that of rabbit pulmonary artery because the former contains α1- adrenoceptors whereas the later has both α1 and α2 adrenoceptors.[13]

Contractions of the rabbit aortic strips caused by potassium chloride depend exclusively on entry of extracellular calcium through the voltage operated calcium channel.[2,14] Such contractions are abolished in the calcium free medium[15] or in the presence of calcium channel blockers. However, contractions of the tissue evoked by noradrenaline are due to influx of calcium through receptor operated channels and the release of calcium from the intracellular stores.[5, 16] Noradrenaline activates the α adrenoceptors distributed on the myoplasmic membrane. This activation evokes an increase in calcium influx across the membrane and also releases the calcium from the tissue sequestered stores.[6,11]

Researchers hypothesized that cyclooxygenase-2 (COX-2) inhibition might improve endothelial function in hypertensive patients by reducing inflammation and oxidative stress. The overall role of COX-2 inhibition in treating hypertension, particularly its effect on outcomes, awaits clarification in larger randomized trials.[17] Vascular smooth muscle such as arterial rings can be used to show not only responses to classical autonomic drugs, but also the more recently discovered role of the endothelium in modulating vascular responses. Vascular endothelium not only acts as a passive barrier between plasma and extracellular fluid but also as a source of numerous potent chemical mediators. These actively control the contraction of underlying smooth muscle as well as influencing platelet mononuclear cell function.

Nonselective NSAIDs may reduce the risk for myocardial infarction (MI) by inhibiting platelet aggregation.[18-20] On the other hand, studies have postulated that COX-2 inhibitors may also reduce cardiovascular risk by inhibiting vascular inflammation, improving endothelial dysfunction, and enhancing coronary plaque stability.[21-23] These effects may

differ among Cox-2 inhibitors. Along with potential differences in blood pressure effects,[24] recent evidence suggests that celecoxib, rofecoxib may differ in their effects on endothelial dysfunction and oxidative stress.[23]

The increase in BP by cycloxygenase inhibitors of intact subjects is mainly due to inhibition of prostaglandins synthesis, antinatriuretic and vasoconstrictor effect as a result of cycloxygenase inhibition. Moreover there are several other factors operative in increasing blood pressure e.g. release of endothelin I, locally released serotonin by platelets, circulating epinephrine and norepinephrine, AVP, angiotensin I and neuropeptide V. It has been observed that aspirin and diclofenac are calcium channel blockers. So is the case with naproxen. In our research diclofenac, piroxicam and celecoxib have caused vasorelaxant effects in rat aortic ring and rabbit portal vein. Calcium channel blocking effect of these NSAIDs may be responsible for this sort of relaxant effect. Factors which cause dilation of blood vessels include increase CO_2, decreased O_2, increased K+, adenosine, lactate, decreased pH, increased local temperature, NO, kinins, prostacyclins, CGPRPα, substance P, histamine, ANP, VIP and decreased discharge of noradrenergic vasomotor nerves. NSAIDs produce the effects through cycloxygenase inhibition, increased prostacyclins is responsible for vasodilation. Large doses of NSAIDs affect calcium influx into smooth muscle in invitro studies. Influx of calcium through cell membrane of smooth muscle is both independent as well as through adrenergic stimulation. The autonomic nerve supply of veins is less dominant as compared to resistant vessels. More dilatation in case of veins may be due to this weak opposing factor i.e. adrenergic innervations.

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References

1. Nanjo, N. (1984) Effect of noradrenaline and acetyl choline on electromechanical properties of the guinea pig portal vein. *Br. J. Pharmacol,* 81: 427-440.
2. Bolton, T.B. (1979) Mechanism of action of transmitters and other substances on smooth muscle. *Physiol Rev,* 59: 606-718.
3. Dacquet, C., Mirranneu, C., and Mirranneu, J. (1987) Effects of calcium entry blockers on calcium dependent contraction of rat portal vein. *Br.J. Pharmacol,* 92: 203-211.
4. Heaslip, R.J., and Rahwan, R.G. (1982) Evidence for the existence of two distinct pools of intracellular calcium in the rat aorta accessible to mobilization by norepinephrine. *J. Pharma Exp Ther,* 221: 7-13.
5. Marriot, J.F. (1988) A comparison of the effects of the calcium entry blockers verapamil diltiazem and flunarizine against contractions of the rat isolated aorta and portal vein .*Br. J. Pharmacol,* 95: 145-154.
6. Daly, C.J., Dunn, W.R., McGrath, J.G., Miller, D.J., and Wilson, V.G. (1990) An examination of the sources of calcium for contractions mediated by post-junctional alpha-1 and alpha-2 adrenoceptors in several blood vessels isolated from the rabbit. *Br. J. Pharmacol,* 99: 253-260.
7. Perry, W.L.M. (1970). Pharmacological experiments on isolated preparations. By the staff of deparment of pharmacology, University of Edinburgh. Churchill Livingstone. Edinburgh: 1-96.
8. Granger, S.E., Holingworth, M., and Weston, A.B. (1985) A Comparison of several calcium antagonists on uterine, vascular and cardiac muscles from the rat. *Br. J. Pharmacol*, 85: 255-262.
9. Dunn, W.R., and Mcgrath, C.J. (1991) Post junctional and adrenoceptors in the rabbit isolated distal sephanous artery indirect sensitivity to parazan of responses to noradrenaline mediated via postjunctional and adrenoceptors. *Br.J.Pharmacol,* 103: 1484-1492.
10. Altura, B.M., Altura, B.T., and Asefa, G. (1980) Differential effects of the calcium antagonist verapamil, on lumen sizes of terminal arterioles and muscular veinules in the rat mesenteric, pial and skeletal muscle microvasculature. *Br. J Pharmacol,* 70: 351-353.
11. Kanmura, Y., Itoh, T., Suzuki, H., Itoh, Y., and Kuriyama, H. (1983) Effects of nifedipine on smooth muscle cells of the rabbit mesenteric artery. *J. Pharmacol. Exp. Ther,* 226 (1): 238-248.
12. Itoh, Y., Kitamra, K., and Kuriyama, H. (1980) Actions of nitroglycerin on the membrane and mechanical properties of stomach muscles of the coronary artery of the pig. *Br. J. Pharmacol,* 70: 197-204.
13. McGrath, J.C., and Wilson, V.G. (1988) Examination of the postjunctional alpha adrenoceptors types for (-) noradrenaline in several isolated blood vessels from the rabbit. *Br. J. Pharmacol*; 95: 473-484.
14. Brading, A.F., and Sneddon, P. (1980) Evidence for multiple sources of calcium for activation of the contractile mechanism of guinea pig taenia coli on stimulation with carbachol. *Br. J. Pharmacol*: 229-240.

15. Hudgins, P., and Weiss, G. (1968) Differential effects of Ca^{+2} removal upon vascular smooth muscle contraction induced Na, Hist. K. J *Pharmacol. Exp. Ther*, 159: 91-97.
16. Godfraind, T. (1976) Calcium exchange in vascular smooth muscle, action of noradrenaline and lanthanum. *J. Physiol*, 260: 21-35.
17. Widlansky, M.E et al. (2003) Short- and long-term CO-X-2 inhibition reverses endothelial dysfunction in patients with hypertension. *Hypertension*, 42: 310-5.
18. Brochier ML. (1993) Evaluation of flurbiprofen for prevention of reinfarction and reocclusion after successful thrombolysis or angioplasty in acute myocardial infarctt-tion. The Flurbiprofen French Trial. *Eur Heart J*, 14: 951-7.
19. Kimmel, S.E., Berlin, J.A, Reilly, M., Jaskowiak, J., Kishel, L., Chittams. J., et al. (2004) The effects of nonselective non-aspirin non-steroidal anti-inflammatory medications on the risk of nonfatal myocardial infarctt-tion and their interaction with aspirin. *J Am Coll Cardiol*, 43: 985-90.
20. Schafer, A.l. (1999) Effects of Nonsteroidal anti-inflammatory drugs on platelet function and systematic hemostasis. *J. Clin Pharmacol*. 35: 209-19.
21. Pitt, B., Pepine, C., and Willerson, J.T. (2002) Cyclooxygenase-2inhibition and cardiovascular events. *Circulation*, 106: 167-9.
22. Van der Wal, A.C., Becker, A.E., van der Loos, C.M., and Das, P.K. (1994) Site of intimal rupture or erosion of thrombosed coronaryt atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation*, 89: 36-44.
23. Hermann, M., Camici, G., Fratton, A., Hurlimann, D., Tanner, F.C., Hellermann, J.P, et al. (2003) Differential effects of selective cyclooxygenase-2 inhibitors on endothelail function in salt-induced hypertension. *Circulation*; 108: 2308-11.
24. Whelton, A., Fort, J.G., Puma, J.A., Normandin, D., Bello, A.E., Verburg, K.M., et al. (2001) Cyclooxygenase -2 specific inhibitors and cardiorenal function: a randomized, controlled trial of celecoxib and rofecoxib in older hypertensive osteoarthritis patients. *Am J Ther*, 8: 85-95.