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VASCULAR SMOOTH MUSCLE, ENDOTHELIAL REGULATION AND EFFECTS OF ASPIRIN IN HYPERTENSION

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1. ABSTRACT

Dysfunction of vascular smooth muscle (VSM) is at the center of occlusive disorders of the cardiovascular system such as hypertension, atherosclerosis, coronary artery disease and hypoxia. In addition to circulating biogenic amines and various neurotransmitters originating from the central nervous system and endocrine system, various autocoids of arachidonic acid metabolism in the blood as well as in the endothelium play an important regulatory role in the maintenance of the tone and the contractile function of VSM. A monolayer of endothelial cells lining the heart and large blood vessels is responsible for producing and releasing both endocrine and paracrine substances such as endothelins, nitric oxide, prostaglandins and prostacyclins. Aspirin, (acetylsalicylic acid/ASA) an ancient remedy against fever and pain, is emerging as an effective drug not only against occlusive disorders but also against various cancers and the AIDs virus. During pregnancy induced hypertension (PIH) and in occlusive disorders, aspirin provides relief through inhibition of cyclooxygenase, an enzyme required for the metabolism of arachidonic acid to produce prostaglandins and prostacyclins in platelets and in endothelial cells. Because of its unique molecular constitution, synergistic ability and solubility in the lipidic environment, various mechanisms of aspirin's actions are being currently investigated. In this review, the effect of aspirin on the regulation of VSM in the presence and absence of endothelium are discussed.

2. INTRODUCTION

Every day, new benefits of aspirin as a wonder drug are being discovered by investigators and clinicians. This review will concern itself with Vascular Smooth Muscle (VSM) regulation via endothelial mediation and will address the direct and indirect effects of aspirin (acetylsalicylic acid) on the contractility of VSM. It is important to understand the mechanism of aspirin action as it is being prescribed increasingly for the management of various occlusive disorders of the vascular system and other pathologic conditions. It is the acetylation and consequent inhibition of the enzyme, cyclooxygenase, by aspirin which renders it so effective in the management of disorders of the vascular system. In addition to platelets, cyclooxygenase is found in the endothelial lining of the heart and the blood vessels.

VSM is regulated by a wide variety of factors including neuropeptides, biogenic amines, prostanoids and endothelium-derived vasoactive substances. The force generated by VSM corresponds

to the concentration of intracellular free calcium ion $[Ca^{2+}]_i$ which is released from intracellular stores or imported from extra cellular pools of Ca^{2+}, through various channels (1). The myocyte relaxes when Ca^{2+} returns to sarcoplasmic stores or is extruded to the extracellular space. Impairment of VSM functions is at the center of an array of cardiovascular pathologies such as arteriosclerosis, coronary heart disease, stroke and hypertension (2). Most of these abnormalities are manifested in an impedance of Ca^{2+} conductance to and from intracellular and extracellular Ca^{2+} stores (3,4). Similarities exist in VSM and striated muscle physiology. However, unlike cardiac and skeletal muscles, many blood vessels remain in a contracted state for long periods of time. There is no refractory period or fatigue in VSM. Also, VSM contraction is mostly tonic in nature. The force maintenance mechanism in VSM termed the "Latch State" is extremely energy efficient (5). Maintenance of the latch state requires Ca^{2+}; however, the levels are considerably lower than those measured during the early phase of activation (6,7,8).

This review will include a short description of the regulation of contraction and relaxation of VSM and the role of Ca^{2+} in this event. The discussion will focus on the role of the endothelium on the conductance of Ca^{2+} in VSM in normotensive and hypertensive conditions. Since, cyclooxygenase in the endothelium is responsible for vasoactive metabolites of arachidonic acid, the effects and interaction of aspirin on the regulatory functions of endothelium will be discussed. Finally, a discussion of some of the direct effects of aspirin on VSM in the absence of endothelium will be considered and the possible mechanisms for such effects will be suggested.

3. REGULATION OF VSM CONTRACTION AND RELAXATION

The contraction and relaxation of VSM is regulated by the $[Ca^{2+}]_i$. Free $[Ca^{2+}]_i$ makes a complex with calmodulin which activates myosin light-chain kinase (MLCK). The activated MLCK phosphorylates LC_{20}, one of the two light-chain subunits of myosin. Phosphorylation of LC_{20} is the signal that activates cycling of actin and myosin cross bridges, which in turn initiates contraction in VSM (1,9). The relaxation of smooth muscle comes about with the decomposition of acto-myosin cross bridges. For this to happen, myosin is dephosphorylated by a phosphatase that is not Ca^{2+} dependent (10). Relaxation of VSM requires re-sequestering of calcium by the sarcoplasmic reticulum (SR). The re-sequestration of calcium is assisted by a Ca^{2+}pump at the plasma membrane (1) and extrusion by a different Ca^{2+} pump (11,12).

4. ENDOTHELIAL MEDIATION

Large blood vessels and the heart are lined by a monolayer of endothelial cells. Under normal conditions endothelial cells are the only cell type that are in direct contact with the blood (13-16). Since Furchgott and Jawadzski demonstrated in 1980 that acetylcholine-evoked relaxation of blood vessels is dependent upon the presence of intact endothelium, it has become increasingly clear that the endothelium plays a major role in the control of regulation of vascular tone, growth, and adhesion (14,17-33). Endothelial cells synthesize vasoconstrictor and vasodilator substances, inactivate circulating hormones, convert inactive precursors into vasoactive mediators and growth factors. The endothelium also responds to flow and /or shear stress, blood borne agents and cells. Consequently, it exerts influence on VSM tone, and plays a role in angiogenesis, VSM proliferation and differentiation (13,14,16,17,21,22,25,27,28, 31,34-36). Both contracting and relaxing types of vasoactive substances are produced and released by the endothelium in blood vessels and affect VSM. Contracting factors of endothelial origin are: a. Three types of Endothelins which are peptides that contain 21-amino acids and two disulfide bridges (30). b. Metabolites of arachidonic acid (AA), prostacyclins and prostaglandins synthesized through the mediation of cyclooxygenase such as thromboxaneA_2 (TXA_2), prostaglandin H_2 (PGH_2), Prostacyclins (PGI_2 ,PHE_2), and prostaglandin F_2 (PGF_2) (15,33,37-39). c. Endothelium-derived contracting factor (EDCF1) during hypoxia (32,38,40) and prostaglandin H_2 also known as endothelium-derived contracting factor 2 (EDCF2) (41). A major vasorelaxant of endothelial origin is endothelium-derived relaxing factor (EDRF) which has been identified as nitric oxide (NO) (15,42). Other known vasoactive substances are a hyperpolarizing factor of unknown nature named endothelium-derived hyperpolarizing factor (EDHF) (43,44) and angiotensin II (ATII) (15,33,45). Additionally, mechanical processes like shear stress due to circulating blood in vessels can up-regulate the nitric oxide synthase gene in endothelium and can also induce VSM relaxation during exercise. The latter response is immediate and is not regulated at the gene expression level, but has been proposed to involve tyrosine kinase activity (15,46,47).

4.1 Endothelins

Endothelins are a powerful family of vasoconstrictors that contain 21 amino acids (30). Analysis of endothelin (ET) genes has revealed the existence of three distinct ETs, designated ET-1, ET-2 and ET-3. Because of structural similarities with certain neurotoxins, ET-1 is thought to activate directly the voltage-operated Ca^{2+} channels (26) and phospholipase C (48), thus bringing about contraction. ET-1 is also reported to activate phospholipase A_2 (48,49). Three receptors, ET_A, ET_B and ET_C, have also been described for ETs on the VSM cells. Of these, ET_A has been identified as the receptor that regulates tone and growth in VSM cells through the action of ET-1 (18,30,48, 50,51). It has been proposed that ET-1 is a mediator of the pressor response to hypoxia and endothelium plays a modulatory role in these responses (40). In porcine arteries, ET-1 was determined to be capable of attenuating its own vasoconstrictive activity, and PGI_2 could attenuate ET-1-induced contraction without altering cAMP levels (52). However, in the rabbit abdominal aorta, ET-1 is reported to stimulate the liberation of vasorelaxant NO (33). In rat aortic rings, ET-1-induced contraction and PGI_2 release maintained in part by Protein Kinase C is due to increased influx of extracellular calcium (49).

Studies have shown that, during hypoxia, ET-1 release is augmented in humans (53). It is thought that endothelium-derived relaxing factor, nitric oxide, and angiotensin-(1-7) mechanisms may act synergistically to buffer the increase in vascular resistance during renovascular hypertension (54). ET-1 was shown to induce the release of prostaglandin H_2, which is also known as EDCF2, from the endothelial cells of rat aorta (41). Increased synthesis of ET-1 is reported during cyclosporin-induced hypertension in rats (55). Isolated rat thoracic aortic rings have been reported to relax in response to ET-3 induced NO release from endothelium via ET_B receptor. As a result of NO release, soluble guanylate is activated which subsequently produces cyclic GMP. The enzyme contributing to NO formation is suspected to be of the calcium-calmodulin-dependent, constitutive type (56). ET-1 in humans is shown to have a transient vasodilatory effect followed by a pronounced constrictive effect when applied luminally. The vasodilator effect also involves the endothelial cyclooxygenase pathway, suggesting that there is a complex interplay of endothelial mechanisms for vasoconstriction as well as vasodilation (35,57).

4.2. Arachidonic Acid Metabolites

Arachidonic acid (AA) metabolites synthesized through the mediation of cyclooxygenase are prostacyclins (PGI_2), prostaglandins PGH_2, PGE_2, and PGF_2 (33,38,58,170). Another AA metabolite synthesized by cyclooxygenase is thromboxaneA_2 (TXA_2). However, the site of TXA_2 synthesis is the platelets. Endothelium-dependent contraction can also be mediated by activation of cyclooxygenase and PGH_2 and TXA_2 receptors (38,41,59,60). The endothelial pathway has been implicated in ET-1 induced vasodilation in humans, suggesting a complex interplay between the vasoactive agents of endothelial origin (57). It has been reported that E-series prostaglandins produce relaxation and at the same time, increase $[Ca^{2+}]_i$ levels in isolated rat aortic muscle, leading to activation of Ca^{2+}-dependent K^+ channels. Very low effective concentrations of PGEs (50-100 nM) support the idea of receptor-activated mechanism in the VSM (61). For the most part, the vascular endothelium functions in a paracrine fashion. However, the endocardial endothelium has been found to produce copious amount of PGI_2 during hypoxia. The spillover from hypoxia-induced endocardial secretion of PGI_2 has been found to affect the vessel muscles downstream from the heart. Thus, endocardial endothelium acts in an endocrine manner in addition to its paracrine effect on myocardium (62). In the endothelium, arachidonic acid metabolism mediated by the enzyme cyclooxygenase is known to be inhibited by acetylsalicylic acid and indomethacin (63-68).

4.3. Nitric Oxide (NO)

Exposure to increased NO desensitizes VSM to vasoconstrictors (69,70) and attenuates the relative rate of protein synthesis in the endothelium and VSM (71). NO is an inhibitor of platelet aggregation and is produced in the endothelium as well as in the plasma membrane of VSM cells via the mediation of the enzyme, NO-synthase (NOS) (30). In the endothelium, NO is synthesized when hormones and autocoids like acetylcholine, bradykinin and substance P act on specific receptors or when the endothelium experiences increased flow and shear stress (15,46,47,72). The release of NO by calcium ionophore is independent of receptor activation (33,34). In rat aortic rings, NO is also reported to be produced in response to ET-3 stimulation (73). It is now well established that NO accounts for all oxygenated nitrogen species such as, dinotrosyl-Fe^{2+} or S-nitrosothiol, synthesized enzymatically from L-arginine (20,34,72, 74-76). NO-Synthase the NO producing enzyme is a reduced NADPH-dependent dioxygenase. Three isozymes of inducible and constitutive types exist as isoform I, II and III (72,77). The inducible isoform II is Ca^{2+}-independent and found mostly in cytokine-activated cells such as macrophages, smooth muscle cells and endothelial cells. The constitutive isoform I and isoform III of NO-synthase are found mainly in neuronal and endothelial cells respectively and are Ca^{2+}/calmodulin dependent (77,78). The constitutive type has a basal level of production in endothelial cells. However, higher levels of the constitutive type can be produced in the endothelium through receptor dependent as well as independent stimulation (72). Human genes for these three isozymes have been localized on chromosome number 12 region 12q24.1 to 12q24.3 for isoform I (79), chromosome number 17 region 17p11-17q11 for isoform II (80) and chromosome number 7 region 7q35-7q36 for the isoform III (81). Endothelial dysfunction associated with aging and hypertension results in reduced production of NO in heart and large blood vessels (30,45,52,77,82). Recently, it was demonstrated that NO from the endothelium, through the mediation of cGMP, inhibits ET-1 activated Ca^{2+}-permeable non-selective cation channels in rat aortic VSM (52,83) and activates both ATP- and Ca^{2+}-dependent K^+ current in small mesenteric arteries of rat (84). Thus, it is clear that NO of endothelial origin is an important agent for the regulation of vascular reactivity.

5. ENDOTHELIUM IN HYPERTENSION

Under normal physiological conditions, the endothelium plays a protective role in the circulation by releasing substances that prevent platelet aggregation, vasoconstriction, and smooth muscle proliferation. During pathological conditions, the protective role of endothelium is diminished and the activity of the contracting factors becomes more pronounced (38). The precise onset of endothelial dysfunction has not been determined yet as acetylcholine and nitroprusside induced relaxation is unimpaired in arteries of young hypertensive (10 to 14 week-old) SHR rats (22). As reviewed by Hughes, in response to increased blood pressure, endothelial cells undergo changes in shape, increase in height, and develop non-uniform nuclei (1). Endothelial cell replication is reported to be increased in aorta. Morphological changes are accompanied by functional changes in the endothelium. Hypertension results in increased permeability of the intima to albumin lipoproteins, horseradish peroxidase and ferritin.

Endothelium-dependent relaxation is attenuated in several animal models of hypertension

both *in vivo* and *in vitro*. This reduction in endothelium-dependent relaxation is associated with a reduced production of NO and a reduced responsiveness to acetylcholine (14,15,38,46,82,85). Aortic smooth muscle cells in Spontaneously Hypertensive Rats (SHR) have been reported to accumulate greater amounts of cGMP than Wistar Kyoto (WKY) cells in response to endogenous or exogenous NO, possibly because of increased levels of soluble guanylate cyclase (86). Elevated levels of ET-1 are observed in hypercholestrolemia, atherosclerosis, pulmonary hypertension, experimental renal hypertension and scleroderma (15,26,30, 50,87). A possible mechanism has been proposed by Chua *et al* (88) for short lived TXA_2 to induce the long lasting ET-1, thus, suggesting a role for ET-1 in the pathogenesis of coronary atherosclerosis and hypertension. However, in both the experimental animal and human hypertension, endothelin levels are not consistently reported to be elevated which can be ascribed to poor methodology and non-specific assays. Therefore, the data on circulating endothelin concentrations are not satisfactory. The influence of endothelin on the pathogenesis of hypertension is not clear (27,89). As reviewed by Calver, *et al* (34) and described by Neild (89), abnormalities of L-arginine-NO system have been identified, although the clarification of mechanisms for these abnormalities awaits further research. Arteriosclerosis and hypertension are reported to accompany an imbalance of growth-regulatory substances of endothelial origin, thereby causing excessive proliferation of VSM cells (71,90). Studies have shown that aortic rings from SHR rats produce larger amounts of prostaglandin H_2 than the WKY control rats and this release is modulated by ET-1 (41). The increased basal tone in aortic coarctation in rat has been shown to be primarily due to PGH_2 and consequent decrease in NO levels (35,91). Hypertension, in combination with aging, has been documented to induce an endothelial dysfunction in conduit arteries (aorta), but not in resistance vessels of the rat (82,92). Pulmonary hypertension in humans is accompanied by endothelial dysfunction, reduced NO levels and accompanied hypertrophy, abnormal proliferation and increased extracellular matrix in vessels (71,72,76). Developmental dynamics of endothelial and neuronal control of thoracic aorta in dogs demonstrated that the vasoactive function of the endothelium is already fully developed in the fetal stage, whereas neuronal control is minimal and becomes increasingly detectable as the puppies grow (93).

It has been suggested that one reason why women of reproductive age have lower incidence of coronary heart disease is the fact that NO release in the endothelium may be modulated by estrogen and estrogen receptor modulators like LY117018 (94). Female SHR rats were reported to produce more EDRF/NO and less EDCF than male rats in aortas. This suggests a less severe endothelial dysfunction in females which could be attributable to the female sex hormone, estrogen (93,95).

6. ASPIRIN AND ITS THERAPEUTIC USAGE

The analgesic properties of willow bark which are due to salicylates were known to cultures as ancient as the Greeks and as isolated as the Indians of North America. Aspirin (acetylsalicylic acid) became available in 1899 through the work of Felix Hoffman at Bayer Industries (96). By 1960, aspirin became the most commonly used medicine for the treatment of pain and fever. The widespread use of aspirin brought to light a serious side effect, gastrointestinal bleeding. This led to an increase in the use of other anti-inflammatory drugs which were less gastrotoxic and nephrotoxic. The evidence that Reye's syndrome was somehow related to the use of aspirin was another negative aspect of aspirin. The discovery, by John Vane in 1971, that aspirin and other non-steroidal anti-inflammatory drugs blocked the synthesis of prostaglandins by cells and tissues and the report by Smith and Willis that inhibition of platelet aggregation by aspirin was related to aspirin's ability to interfere with prostaglandin synthesis in the platelets (67,68) led to a complete change in the reputation of aspirin.

Acetylsalicylic acid, (ASA/aspirin) has been shown to prevent pregnancy induced hypertension (PIH) in humans (48,97-106), and low dosages of ASA are prescribed to manage occlusive disorders like angina, myocardial infarction and to prevent re-stenosis (9,13,56, 58,63,75,86,97,102,107-124). Aspirin has also been found to be beneficial against vascular disorders associated with diabetes (9,110,125). Aspirin has found a therapeutic use against several types of cancers of digestive tract (18,105, 126,127), and AIDS (128). Recently, aspirin was found to have neuroprotective properties (125,128-130).

The beneficial effect of ASA during PIH and occlusive vascular disorders has been ascribed to its inhibition of the synthesis of the vasoconstrictor, TXA_2. TXA_2 is produced in blood platelets from arachidonic acid by cyclooxygenase. In the endothelium, cyclooxygenase converts arachidonic acid to prostacyclins which are vasodilators. One of the prostacyclins, namely PGI_2 is stable enough to elicit hypotensive action (39). Low doses of aspirin are known to inhibit cyclooxygenase in platelets, but not in the endothelium, thus, preventing PIH (39,97-102,104-106,131). A great deal of work is being conducted to investigate and understand various mechanisms through which aspirin provides relief in so many different metabolic and pathologic disorders. Recently, it was demonstrated that the therapeutic efficacy of aspirin is enhanced when it is administered in association with zwitterionic phospholipids such as dipalmitophos-phatidylcholine (DPPC). Several molecular mechanisms are being examined to explain this DPPC-dependent increase in aspirin's antipyretic, anti-inflammatory and analgesic activity observed in rodent model systems (65,132-134).

In the body, ASA is metabolized within 6 hours and the physiological concentrations of 0.2-0.6 mMole in the blood of subjects taking the drug have been reported (125). Two esterases called aspirin esterase-I and esterase-II have been identified in mice, rats and humans. These esterases promote the metabolism of aspirin by hydrolysis to salicylic acid (135-137).

The analgesic and antinociceptive effects of ASA have been ascribed to ASA mediated changes in firing discharges in brain areas associated with the release of 5-hydroxytryptamine (serotonin) and 5-hydroxyindole acetic acid, a metabolite of serotonin (125), and thus establishing its involvement in serotonergic and opioid pathways (138). Aspirin is considered to be a mild analgesic because it interferes with the production of prostaglandins which are known to accentuate rather than mediate pain producing activity of agents such as bradykinin and 5-hydroxytryptamine (139).

As described by Lecomte, *et al* (64) there are two isozymes of prostaglandin endoperoxide synthase, PGHS-1 and PGHS-2 that exhibit both cyclooxygenase and endoperoxide synthase activities, respectively (140). Cyclooxygenase-1 is expressed constitutively and cyclooxygenase-2 is expresses as an immediate early response to growth factors, cytokines and tumor promoting factors (140). Cloning of cDNA and mutagnesis studies of these isozymes have shown that inactivation of cyclooxygenase-1 is brought about by its acetylation at amino acid residue ser-530 and that of cyclooxygenase-2 at ser-516 by ASA (64).

The tumor or cancer preventing mechanisms of action of ASA are not yet well characterized. Most of the positive effects have been determined as the result of population or randomized trial studies (17,126,127,140). In a study (126) involving a BJ6 mouse epidermal cell line, it was reported that aspirin and aspirin-like salicylates inhibit transcription factor protein 1 (AP-1). This inhibition of AP-1 did not involve the prostaglandin or the mitogen-activated protein kinases, Erk 1 and Erk 2 pathways. This study also reported that H^+ ion pump inhibitor diethylstilbestrol (DES) caused a dose-dependent inhibition of AP-1 activities which led to the hypothesis that elevated intracellular H^+ concentrations may be responsible for inhibition of AP-1 in this mechanism (126). Based on the fact that ASA and other non steroidal anti-Inflammatory drugs (NSAIDs) inhibit tumor induced ornithine-decarboxylase activity, it was proposed that prostaglandin E_2 may be a mediator for reduced morbidity from colon cancer with aspirin treatment (18,33).

Aspirin was found not to increase the risk of bleeding during macular degeneration (141) and, a Nurses Health Study (118), showed no increase in the risk for cataract formation. Recently, beneficial effects of ASA have been reported in HIV infection (128). Aspirin, because of its ability to inhibit cyclooxygenase, seems to have a considerable effect on hypothalamic controlled and released hormones such as GnH, LH and ACTH via the opiatergic and cholinergic pathways (142). Aspirin is reported to severely curtail the production of prostaglandins in the gall bladder without any decrease in its contractility or stone formation (143). Both beneficial and adverse effects of ASA upon the immune responses have also been documented in the literature (18,131,144-147).

Aspirin has been reported to release significantly more prostaglandin F-alpha into the plasma in aspirin sensitive asthma patients than non-sensitive patients (147). Serum tryptase levels of patients who were desensitized to ASA were not increased indicating that mast cell (MC) activation may be caused by aspirin in aspirin-sensitive asthma patients (144,148). A double blind placebo controlled study has suggested that aspirin is associated with suppression of antibody response which may be mediated via its effect on monocytes or mononuclear phagocytes (145,146).

There are numerous reports of enhanced efficacy in a myriad of its therapeutic functions when aspirin is used in conjunction with other pharmacological agents. For example, a phospholipid (DPPC)-induced increase in anti-inflammatory and anti-pyretic activities is suggested to be due to accelerated diffusion of aspirin across lipidic membranes and into target cells at the neutral pH of the blood (134). Alternatively, the aspirin-DPPC complex has an increased affinity to bind to and inhibit specific isoforms of cyclooxygenase that catalyze the rate limiting steps in prostaglandin synthesis. A possible third mechanism may be that the chemical association with DPPC increases the half life of aspirin by reducing its conversion to salicylic acid (65). Anti-thrombotic activity of aspirin is enhanced when it is used in conjunction with dipyridamole (9,110). Reduced platelet deposition on the sub-endothelium is observed when ASA is administered together with Ticlopidine which is an indication of reduced aggregability and thrombotic activity (119). In anti-thrombotic therapy, the inclusion of limolenne in an aspirin patch has been proposed to reduce gastric toxicity and to improve permeability for drug delivery through the skin (149). In a randomized controlled trial, the use of ketanserin and aspirin in combination was found to be more effective against pre-eclampsia and severe hypertension than aspirin alone (150). A phenolic compound, trans-resveratal, found in red wine, was recently reported to have added an inhibitory effect on platelet aggregation via reduced serotonin production which does not involve the prostaglandin pathway (121).

Recently, it was reported that therapeutic concentrations of aspirin do not interfere with transcriptional processes. However, at high concentrations of 10-20 mM, aspirin does interfere with the transcriptional machinery. This was shown by the failure of [^{35}S]-methionine incorporation into total rat islet proteins and by inhibition of rabbit reticulocyte expression by Bromo mosaic virus mRNA (151). ASA is reported not to interfere with the cardioprotective agent ifetroban, which is a potent and selective antagonist for the thrombaxane/prostaglandin endoperoxide receptor (117).

6.1. Role of Cyclooxygenase in the Pharmacology of Aspirin

Pregnancy-induced hypertension (PIH) in humans has been shown to be prevented by ASA administration (48,97-102,104-106). Increasingly low aspirin dosages are being prescribed to manage and prevent various occlusive disorders such as angina, myocardial infarction and re-stenosis after angioplasty

(58,63,97,101,113,115, 116,120, 123,131,152). Aspirin reduces coronary hyperreactivity to autocoids aftert angioplasty and thus prevents re-stenosis (56,58,109,153). Aspirin, together with other NSAIDs, has been suggested to inhibit the growth of colon tumors and to decrease the mortality rate from cancer of the colon in humans (18,105,126,127,140). Recently, ASA was found to suppress epithelial cell proliferation in rat colonic crypts (17). The beneficial effects of aspirin in vascular occlusive disease and PIH are ascribed to its blockade of the enzyme, cyclooxygenase, during the synthesis of platelet TXA_2, a potent agonist of vascular smooth muscle contraction (37,60,63,75,108,109,112,115,116,120,122,123,139, 150, 152, 154,155). Indomethacin, another cyclooxygenase inhibitor has been found to depress norepinephrine-induced constriction in rat abdominal aorta, but not in thoracic aorta, thus, indicating that norepinephrine constriction is mediated by a constrictor prostanoid of VSM origin that is different from TXA_2 (156-158). ASA has been reported to induce alterations in membrane proteins and to induce reorganization of lipid assembly which, in turn, brings about conformational changes in membrane proteins in blood cell membranes (125). As cited by Watala (125), aspirin was found to cause alterations in alpha-2-adrenoceptors of platelets (159). More recently, pre-treatment with acetylsalicylic acid is reported to improve post-hypoxic recovery of neuronal function and neuroprotection (130,160).

Following the publication of contradictory reports concerning the effects of prostaglandins on the contractility of vessels in SHR and experimental hypertensive rats, by Yin, *et al* (161) and Roson, *et al* (66), effects of cyclooxygenase inhibition with ASA and indomethacin on rat aortic ring contractility were evaluated and reported (157,158,162). A brief account of these and some additional studies from author's laboratory are described below.

6.1.1. Effects of Cyclooxygenase Inhibition in SHR and WKY Rats

The effects of cyclooxygenase inhibition on the contractile response of aortic smooth muscle to $alpha_1$- and $alpha_2$-agonists were investigated in male and female SHR animals. Indomethacin and acetylsalicylic acid (ASA) were used as inhibitors of cyclooxygenase. Exposure durations of 10, 20 and 30 minutes to 0.2 mM ASA prior to eliciting contractions with KCl, PE and NE did not alter the intensity of contractile responses which is contrary to the reported results of the inhibition of contractile response due to ASA (66).

6.2. Effects of Aspirin on Vasoreactivity

Effects of ASA on the aortic vascular smooth muscle contractility were assessed both in male and female SHR and WKY rats. These effects were studied both for *in vitro* (0.2mM) as well as*in vivo* (10 mg/kg) administration of the drug. For the *in vivo* administration, two routes were employed, IP injections and inclusion of aspirin in the drinking water. The contractile responses of aortic rings were measured with and without endothelium. Also, experiments were conducted to evaluate the effects of ASA on intracellular Ca^{2+} release as well as its import from the extracellular gradient.

6.2.1. *In vitro* Effects

6.2.1.1. Vasoreactivity of aortic rings with intact endothelium

A comparison of the dose response curve for Phenylephrine (PE) by aortic rings with intact endothelium from SHR and WKY rats in the presence or absence of 0.2 mM ASA with a Krebs solution bathing the tissues showed that aortic rings from SHR animals produced significantly higher active tension in the range of 10^{-8}-10^{-4} M PE concentration than the rings without ASA treatment. The active tension of rings from WKY animals was not affected by ASA. The calculated ED_{50} of PE for SHR rings in the presence of 0.2mM ASA was 10^{-7} M; for the rings without ASA treatment the ED_{50} was $5x10^{-7}$M (157). These observations were consistent in both male and female rats.

6.2.1.2. Vasoreactivity of Aaortic Rings Denuded of Endothelium

The removal of endothelium generally rendered the rings more responsive to lower concentrations of KCl. In the presence of ASA, the dose response curve to cumulative concentration of KCl was significantly higher in denuded rings than in non-denuded rings. However, the enhancement of response to the maximal dose of KCl in denuded rings of SHR did not equal the normal response of WKY control rats. Both in male and female rats, the denuded rings from SHR animals produced significantly higher active tension in the presence of ASA in response to $alpha_1$-adrenoceptor stimulation by PE concentrations of 10^{-7}-10^{-4} M than did the intact rings. The calculated ED_{50} for PE in the presence of ASA was approximately $8x10^{-7}$ M which is one log less than in the absence of ASA (157) indicating a positive effect of aspirin on the efficacy of $alpha_2$-adrenoceptors. Identical results were obtained with aortic rings from female rats.

6.2.2. *In Vivo* Effects

6.2.2.1. Intraperitoneal Administration of ASA and Aortic Contractility in Female SHR Rats

This study was designed to investigate the effects of *in vivo* administration of ASA on the blood pressure in female SHR rats and contractility of aortic smooth muscle in response to KCl, a non-specific membrane depolarizing agent and $alpha_1$- and $alpha_2$-adrenergic stimulation using agonists such as PE, clonidine and NE (158). Young adult SHR and WKY rats were administered intraperitoneal injection (IP) of 10 mg/kg ASA twice weekly for three weeks. Systolic blood pressure of each animal was monitored two times during each week of drug administration.

The maximal KCl-invoked contractile responses of rings from SHR controls were significantly less than the maximal response of rings from WKY controls. However, the rings from ASA-treated SHR animals produced contractions either equal to or exceeding the contractions produced by healthy WKY controls. The contractile responses of rings from ASA-treated SHR animals were significantly enhanced as compared to those of rings from the

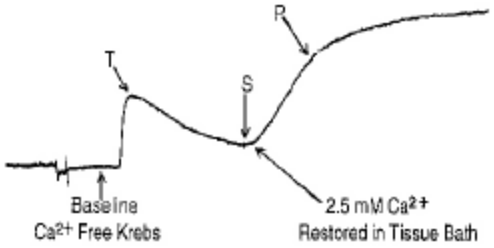

Figure 1.Transient (T) contraction in response to 10^{-6}M PE in Ca^{2+} free Krebs decays to a sustained (S) component within 2 minutes. When extracellular gradient of Ca^{2+} is restored by injecting 2.5 mM Ca^{2+}, a much larger phasic (P) contraction is elicited as a consequence of Ca^{2+} import from the extracellular spaces.

non-treated SHR animals in the range of 20-80 mM KCl. The PE induced $alpha_1$-adrenoceptor stimulated aortic contractions due to ASA treatment of SHR rats were restored to the level of active tension that was produced by aortic rings of non-hypertensive WKY controls. Clonidine, a preferential $alpha_2$-agonist, invoked similar aortic responses from ASA treated SHR as well as non ASA-treated WKY control female rats which were significantly higher than the responses generated by non-treated SHR. It was interesting to note that ASA treatment showed gender differences for its effect on $alpha_2$-adrenoceptor mediated contraction, aortic rings from female SHR rats generated significantly higher responses than male SHR rats to clonidine. Pharmacologic doses of NE, a potent generalized alpha-agonist, in the concentration range of 10^{-6}-10^{-4} M, elicited significantly higher tensions in ASA treated SHR rats than the non-ASA treated SHR rats. However, these enhanced contractions did not equal to those generated by aortic rings from control WKY animals (158).

6.2.2.2. Effect of *in vivo* ASA Administration on Aortic Contractility and the Blood Pressure in Male SHR Rats

In these experiments, ASA was administered intraperitoneally, and in drinking water to different groups of male rats . During the ASA treatment period, blood pressure was monitored and, at the end of drug administration period, aortic rings were assessed for their release as well as import of Ca^{2+} as measured by their contractility in response to Ca^{2+} from these reservoirs.

The maximal response to KCl (8-80 mM) from rings of SHR controls (no ASA treatment) was significantly less than the maximal response of rings from WKY controls. However, the rings from ASA-treated SHR animals produced contractions significantly exceeding those of non-ASA-treated SHR controls in the range of 20-80 mM KCl. The responses of rings from ASA-treated WKY rats were somewhat depressed when compared to the non-treated WKY controls. Significantly higher active tensions were induced by PE in aortic rings from ASA-treated SHR rats than by the aortic rings from the non-treated SHR rats. ASA treatment however, did not affect significantly the responses of WKY control rats (158).

Administration of ASA, either IP or in the drinking water did not affect $alpha_2$-agonist clonidine induced aortic contractions in male SHR rats. Both ASA treated and non-treated WKY rats produced significantly higher contraction in response to $alpha_2$-adrenoceptor stimulation via clonidine. However, the responses of ASA treated WKY rats were suppressed in the pharmacologic concentrations of 10^{-6}-10^{-4} M. This indicates that $alpha_2$-adrenoceptor stimulation in male SHR is not modulated by any of the processes or mechanisms that ASA affects in the VSM or in the endothelium (162).

6.2.2.3. Ca^{2+} Conductance as Measured by Active Tension Generated By Aortic Rings

Figure 1 is an actual recording for the Ca^{2+} conductance protocol. Adrenergic stimulation with 10^{-6} M $alpha_1$- specific agonist phenylephrine in calcium-free Krebs solution caused the release of Ca^{2+} from intracellular stores to produce a transient (T) contraction which decayed rapidly to a residual sustained (S) contraction. The addition of 2.5 mM Ca^{2+} to the tissues bathing in Krebs solution resulted in the import of Ca^{2+} from extra cellular spaces, causing an increase in the concentration of intracellular free calcium $[Ca^{2+}]_i$ and further enhanced the active tension generated by the vascular smooth muscle. This latter response was used to assess the Ca^{2+} import mechanisms through the cell membrane. The phasic contraction due to the import of extracellular calcium is reported to have two components. One component is under the control of IP_3 cascade and protein kinase C. The other component is dependent o Ca^{2+} import from the extracellular stores through the nifedipine sensitive Ca^{2+} channels (163). The results of the protocols to evaluate effects of ASA treatment, and absence or presence of endothelium at the time of adrenergic stimulation of aortic rings from SHR and WKY rats are presented in figure 2. A reference contraction response by rings from various groups of animals was generated to a concentration of 10^{-6} M PE. The results presented in figure 2 shows that the DND rings from SHRs that were administered ASA produced an active tension comparable to the intact rings from WKY controls (rats that were not administered ASA). Also, the intact rings from non-treated (-ASA) SHR animals produced the least active tension of all groups. Similar patterns were observed for the transient (T) and sustained (S) contractions in response to release of internally stored Ca^{2+} alone. There were no significant differences among transient and sustained contraction in rings from ASA-treated SHRs. Upon the restoration of extracellular Ca^{2+}, aortic rings from all groups with the exception of SHR(-ASA) cEndo (*i.e.* the non-ASA-treated SHR rings containing endothelium) produced phasic contractions comparable to the reference tension. The response of non-ASA-treated SHRs with intact endothelium was slightly higher than their response for the reference contraction. Significantly higher contractile response to adrenergic stimulation was elicited by the DND rings from ASA-treated SHRs under normal concentrations of internal in response to intracellular calcium and external Ca^{2+} when compared

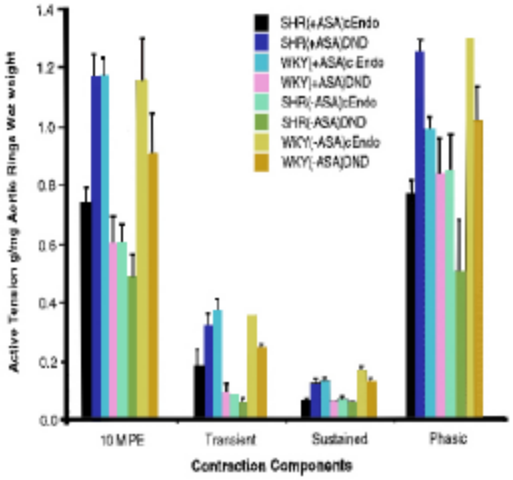

Figure 2. A comparison of the active tension produced release and due to calcium import from extra-cellular spaces in endothelium denuded and non-denuded SHR and WKY rat aortic rings that were administered ASA for ten weeks . The data represents means of 8 rings for each group of animals. The hats represent ±SEM.

with rings that contained intact endothelium (cEndo). They produced phasic contraction equal to that of the reference tension. The non-ASA-treated intact rings with endothelium produced slightly higher response than their reference contraction. The rings with ablated endothelium from ASA-treated SHR animals produced 63.7% more contraction than rings with intact endothelium. The minimal contraction in response to Ca^{2+} imported from extracellular spaces was observed in DND rings from non-treated SHRs (115). These results clearly show that ASA enhances the contractile responses of aortic rings without the endothelial mediation in rats. It was also noted that because of its ability to induce alteration in membrane protein conformation and corresponding fluctuations of lipid fluidity during *in vitro* incubation as noted in platelet membranes (125), ASA may be able to correct the defects in the permeability of VSM plasma membrane associated with hypertension (157,158,164,165).

6.2.2.4. Effect of ASA on Systolic Blood Pressure

Aspirin did not have any effect on the course of blood pressure development either in SHR or WKY animals. The route of ASA administration, for that matter, did not make any difference either. Although, we observed a slight initial lag in the rate of blood pressure rise in ASA treated SHR animals, subsequent to 5 weeks of treatment, the treated animals had relatively higher blood pressure than the non-treated WKY animals (115,1157).

7. SIGNIFICANCE

Since $alpha_1$-adrenergic receptors are the predominant type of adrenergic receptors in the thoracic dorsal aorta of the rat (166-168), phenylephrine, a preferential $alpha_1$-adrenergic agonist, was employed in eliciting the contractile response in these experiments. A concentration of 0.2 mM ASA has been reported to inhibit cyclooxygenase in *in vitro* procedures (66). It is a well established fact that aortic contractility is significantly impaired in SHRs (167-169). In our experiments, IP injection of ASA for three weeks in female SHR animals restored the contractile response of aortic smooth muscle to the levels exhibited by non-ASA treated females WKY control animals(158). In that study, we observed that ASA, *in vitro,* enhanced aortic contractility in response to PE in SHR rats. In WKY rats however, the adrenergic responses were not affected. This enhanced responsiveness can not be attributed to interaction of ASA with platelets and the consequential decrease in the production of TXA_2 by platelets, because these responses were elicited *in vitro*. The involvement of PGI_2 and other EDRFs is also discounted as ASA was able to enhance the contractility in denuded rings from SHR animals. As noted by Schiff *et al* (52), synthesis of cyclic endoperoxidases from arachidonic acid is reduced when cyclooxygenase is inhibited. Accordingly, selective inhibition of platelet-derived cyclooxygenase due to low dosages of aspirin may inhibit cyclooxygenase activity in platelets, but not in the endothelium, thereby diminishing the synthesis of thromboxane and not of prostacyclins in the endothelium. In our experiments, both these avenues of cyclooxygenase-mediated synthesis were effectively eliminated. The ED_{50} for PE did not show marked differences for ASA treated or non-treated rings from SHR animals. An analysis of ED_{50} indicated that the contractile ability of aortic smooth muscle is not fully restored with ASA alone. The enhanced responsiveness of aortic smooth muscle observed in these studies suggests that ASA either affects the regulation of intracellular free calcium ion $[Ca^{2+}]_i$ through $alpha_1$-adrenoceptor gated calcium channels, or ASA influences prostaglandins, especially prostacyclin (PGI_2) which modulates vascular reactivity. The latter conclusion is not in agreement with the results of Roson *et al*. (66) who concluded from their studies on a two kidney-two clip hypertension protocol that prostaglandins do not play any significant role in altered vascular reactivity in hypertensive rats. However, we have observed that indomethacin significantly decreased the responsiveness of aortic tissues to PE in female SHR animals, which strongly suggests that cyclooxygenase inhibition in aortic endothelium modulates VSM contractility in rat.

The SHR animals used in experiments designed to investigate the involvement of paracrine products of endothelium were two weeks younger than the animals employed for comparison of SHR and WKY vascular reactivity. This difference in age between the two groups is not of any consequence, because it is known that the SHR animals have reduced aortic reactivity as compared to WKY from the onset of hypertension which is at the age of six weeks (167-169,171).

These studies strongly suggest that ASA could affect aortic contractility that involves pathways other than the metabolism of arachidonic acid. Since ASA is soluble in lipids, it most probably corrects, to some extent, the defect in permeability of plasma membranes associated with hypertension by bringing

the rigidity back to the plasma membrane. This mode of action for ASA is more plausible since it has been found to change the conformation of plasma proteins when incubated *in vitro* with platelet plasma membranes (125).

Since ASA is lipid soluble, the *in vitro* treatment of aortic rings with ASA may bring about conformational changes in one or several of the plasma membrane proteins such as adrenergic receptors, ion channels or exchangers. The same might be true for sarcoplasmic channels and pump proteins. The enhanced contractility in aortic rings denuded of their endothelium from rats that were given ASA either by injection or in drinking water for extended periods of time, can not be explained based on the lipid solubility of ASA. The half life of ASA in physiological systems (in animal or human body) is about six hours, and the drug is eliminated from a physiological system in less than twenty four hours. Therefore, the enhanced contractility observed in the *in vivo* treated aortic rings suggests a longer lasting effect of ASA on the conductance mechanisms of calcium.

The complex nature of endothelial participation and the role of AA metabolites in the regulation of VSM tone are abundantly evident from the above account and the literature sited. Yet, much work needs to be done to fully understand the interplay between the endothelium and VSM in normal and pathophysiological conditions. To answer the questions "How can aspirin, a cyclooxygenase inhibitor, in the absence of endothelium which is the main source of vasoactive metabolites of arachidonic acid, augment the alpha-adrenoceptor mediated aortic ring constriction" and "What role does the administration of ASA play in, and during, the development of genetic hypertension in SHR rats", more research needs to be done.

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9. REFERENCES

1. David R. Hathaway, Keith L. March, Joseph A. Lash, Leonard P. Adam, and Robert L.Wilensky: Vascular Smooth Muscle, A review of the Molecular Basis of Contractility. *Circulation* 83(20), 382-390 (1991)

2. R. A. Murphy, J. Herlihy, T. Jeremiah, and J. Megerman: Force-Generating Capacity and Contractile Protein Content of Arterial Smooth Muscle. *J Gen Physiol* Vol. 64, 691-705 (1974)

3. D. O. Levitsky, M. Clerguw, F. Lambert, M. V. Souponitskaya, T. H. Le Jemte, Y. Lecarpentier and A. M. Lompre': Sarco[lasmic Reticulum Calcium Transport and Ca^{2+}-ATPase Gene Expression in Tjoracic and Abdominal Aortas of Normotensive and Spotaneously Hypertensive Rats. *J B iol Chem* 268(11),8325-8331, (1993)

4. G. R. Monteith, E. P. Kable, T. H. Kuo, and Roufogalis: Elevated Plasma Memebrane and Sarcoplasmic Reticulum Ca^{2+} Pump mRNA Levels in Cultured Aortic Smooth Muscles from Spontaneously Hypertensive Rats. *Bico Biop R* 230, 344-346, (1997)

5. C-M. Hai, R. A. Murphy: Regulation of shortening velocity by cross-bridge phosphorylation in smooth muscle. *Am J Physiol* 255,C86-C94, (1988)

6. C. M. Rembold, R. A. Murphy: Myoplasmic [Ca^{2+}] determines myosin phosphorylation in agonist-stimulated swine arterial smooth muscle. *Circul Res* 63,593-603, (1988)

7. F. Rioux, and B.A. Berkowitz: Role of the Thyroid Gland in the Development and Maintenance of Spontaneous Hypertension in Rats. *Circul Res* 40(3), 306-312 (1977)

8. R. Shibata, S. Morita, K. Nagi, S. Miyata and T. Iwasaka: Calcium dependence of ouabain-induced contraction in aortas from spontaneously hypertensive rats. *Eur J harmacol* 190, 147-157, (1990)

9. John A. Colwell. Aspirin Therapy in Diabetes: *Diabetese Care* 20(11), 1767-1771, (1997)

10. C. H. Hennekens, M. L. Dyken, and V. Fuster: Aspirin as a Therapeutic Agent in Cardiovascular Disease (AHA Scientific Statement) *Circulation* 96,2751-2753, (1997)

11. E. Carafoli. Calcium Pump of the Plasma Membrane: *Physiol Rev* 71(1); 129-153, (1991)

12. N. Morel, N. Wibo, and T. Godfraind: A calmodulin stimulated Ca^{2+} Pump in rat aorta plasma membranes. *Biochim Biophys* 644,82-88, (1981)

13. Henderson, A. H. Endothelium in Control: *Br Heart J* 65,116-125, (1991)

14. A. D. Hughes, and M. Schachter: Hypertension and Blood Vessels. *Brit Med B* 50(2),356-370, (1994)

15. T. Luscher, and G. Noll: Endothelium as an end-point in interventional trials, Concepts, methods and current data. *J Hypertensi*14 (Suppl 2),S111-121, (1996)

16. S. M. Marchenko, and S. O. Sage: Electrical Properties of Resting and Acetylcholine Stimulated Endothelium in Intact Rat Aorta. *J Physiol* 462,735-751, (1993)

17. C. J. Barnes, W, E. Hardman, and I. L. Cameron: Aspirin, Age, and Proximity to Lymphoid Nodules Influence Cell Proliferation Parameters in Rat Clonic Crypts. *Cell Prolif* 28,59-71, (1995)

18. John, A. Baron, and Robert E. Greenberg: Could Aspirin Really Orevent Colon Cancer. *N Eng J of Medi* 325(23),1644-1646, (1991)

19. D.A. Cox, and M.L. Cohen: Relationship between Phospholipase D Activation and Endothelial Vasomotor Dysfunction in Rabbit Aorta. *J Pharmacol ExpTher* 288(1),305-311, (1997)

20. R. Davisson, J. N. Bates, A. K. Johnson, and S.J. Lewis: Use-Dependednt Loss of Acetylcholine- and Bradykinin-Mediated Vasodilation After Nitric Oxide Synthase Inhibition, Evidence for preformed stores of nitric oxide-containing factors in vascular endothelial cells. *Hypertension* 28,354-369, (1996)

21. Keith A. Freeman, A. Mao, Leif O. Nordberg, J. Pak, and Ronald, J. Tallarida: The Relationship Between Vessel Wall Tension and the Magnitude and Frequency of Oscillation in Rat Aorta. *Life Sci* 56(6)-129-134, (1995)

22. L. C.Fuchs, D. Nuno, K. G.Lamping and , A. K. Johnson: Charaterization of Endothelium-Dependent Vasodilation and Vasoconstriction in Coronary Arteries From Spontaneously Hypertensive Rats. *Am J Hypertens* 9, 475-483 (1996)

23. H. Jahan, S. Kobayashi, J. Nishimura and , H. Kanaide: Endotheliun-1 and angiotensin II act as progression but nit competence growth factors in vascular smooth muscle cells. *Eur J Pharmacol* 295,261-269, (1996)

24. C. Janaskul, R.G. King and , A. L. A. Boura.: Effects of Endothelial Cell Removal on alpha$_1$-Adrenoceptor-Mediated Response of Aorta of Pregnant Rats. *Clin Exp Ph* 17,147-157, (1990)

25. C. Katnik and D. J. Adams: Characterization of ATP-sensitive potassium channels in freshly dissociated rabit aortic endothelial cells. *Am. J. Physiol* 272, H2507-H2511, (1997)

26. Thomas F. Luscher: Endothelin. *J cardio Ph* 18 (Suppl 10),S15-S22, 1991.

27. P. Nava, T. Collados, F Masso and V. Guarner: Endothelin Mediation of Insulin and Glucose-Induced Changes in Vascular Contractility. *Hypertension* 30,825-829, (1997)

28. M. Ohno, J. P. Cooke, V. J. Dzau and G. H. Gibbons: Fluid Shear stress Induces Endotjelial Transforming Growth Factor Beta-1 Transcription and Production. *J Clin. Invest* 95,1363-1369, (1995)

29. V. Richard, M. Hogie, M. Clozel, B-M. Loffler and C. T. Thuillez: In Vivo Evidence of an Endothelin-Induced Vasopressor Tone After Inhibition of Nitric Oxide Synthesis in Rats. *Circulation* 91,771-775, (1995)

30. E. L. Schiffrin: Endothelin, Potential Role in Hypertension and Vascular Hypertrophy, *Hypertension* 25,1135-1143, (1995)

31. S. Seewald, A. Sachinides, R. Dusing, Y. Ko, , C. Seul, P. Epping and H. Vetter: Lysophosphatidic acid and intracellular signalling in vascular smooth muscle cells. *Atherosceler* 130,121-131, (1997)

32. John T Shepherd,. and Zvonimir, S. Katusic: Endothelium-Derived Vasoactive Factor-, I, endo thelium-dependent relaxation. *Hypertension* 18 [Suppl III],III-76-III-85, (1991)

33. S. Suzuki, J. Kajikuri, A.Suzuki and , T. Itoh: Effects of Endothelin-1 on Endothelial Cells in the Porcine Coronary Artery. *Cir Res* 169,1361-1368, (1991)

34. A. Calver, J. Collier and , P. Vallance. Nitric Oxide and Cardiovascular Control. *Exp Physiol* 78,303-326, (1993)

35. C. F. Kung, and , T. F. Luscher: Different Mechanisms of Endothelial Dysfunction With Aging and Hypertension in Rat Aorta. *Hypetension* 25 194-200,(1995)

36. Arthur M. Melkumyants. Balashove, Sergey A. and Kartamyshev, Sergy P: Anticonstrictor Effect of Endothelium Sensitivity to Shear Stress. PFLUGERS ARC; *Eur J Physiol* 427,264-269, (1994)

37. S. S. Bhagwat, P. R. Hamann, W. E. Still, S. R. Bunting and , F. A. Fitzpatrick: Synthesis and Structure of Platelet Aggregation Factor Thromboxane A_2. *Nature* 315,511-513, (19985)

38. Zivonimir S. Katusic, and John T. Shepherd: Endothelium-Derived Vasoactive Factor,II; Endothelium-Dependent Contraction. *Hypertension* [Suppl. III], III-86-III-92, (1991)

39. C. R. Pace-Asciak, M. C. Carrara, G. Rangraj, and , K.C. Nicolaou: Enhance Formation of PGI_2, A Potent Hypotensive Substance, By Aortic Ring and Homogenates of the Spontaneously Hypertensive Rat. *Prostaglandin* 15(6),1005-1012,(1978)

40. Y. Wang, Y. Coe, O. Toyoda and , F. Coceani: Involvement of Endothelin-1 in Hypoxic Pulmonary Vasoconstriction in the Lamb. *J Physiol* 482(2),421-434, (1995)

41. H. Asano, K. Shimizu, , M. Muramatsu, Y. Iwama, Y. Toki, Y., Miyazaki, K. Okumura, H. Hashimoto and T. Ito: Prostaglandin H_2, as an Endothelium-Derived Contracting Factor Modulates Endotheli-1-Induced Contraction. *J Hypertens* 12,383-390, (1994)

42. Ikuko Sato and S. Murota: Paracrine Function of Endothelium-Derived Nitrixc Oxide. *Life Sci* 56(13),1079-187, (1995)

43. M. Fukao, Y. Hattori, M. Kanno, I. Sakuma and A. Kitabatake: Sources of Ca^{2+} in relation to generation of acetylcholine-induced endothelium-dependent hyperpolarization in rat mesenteric artery. *Br J Pharamcol* 120,1328-1334, (1997)

44. C. J. Garland, F., Plane, B. K. Kemp and T. M. Cocks: Endothelium-dependent hyperpolarization, a role in the control of vascular tone. *Trens Phar* 16,23-30, (1995)

45. M. Barton, F., Cosentino, Ralf P. Brandes, P. Moreau, S. Shaw and Thomas F. Luscher: Anatomic Heterogeneity of Vascular Aging, Role of Nitric Oxide and Endothelium *Hypertension* 30,817-824, (1997)

46. I. Fleming, B. Fisskthaler and . Busse: Calcium signaling in ednothelial cells invoves activation of tyrosin kinases and lead to activiation of mitogenic-activated protein kinases. *Cir Res* 76,522-529, (1995)

47. J.E. Gage, O.M. Hess, T. Murakami, M. Ritter, J. Grimm, and H.P. Krayenbuehl: Vasoconstriction of stenotic coronary arteries during dynamic excercise in patients with classic angina pectoris, reversibility by nitroglycerin. *Circulation* 73,865-876, (1995)

48. T. Masaki and Yanagisawa.: Physiology and Pharmacology of Endothelins. *Med Res Rev* 12(4),391-421, (1992)

49. G. K. Oriji and H. R. Keiser: Role of Calciuym in endothelin-induced cintraction and prostacyclin release. *Prostagland* (*Leukotrienes and Essentail Fatty Acids)* 55(6),413-417, (1996)

50. B. Battisini, P. Chailler, P. D'Orleanse-Juste, N. Briere and P. Sirois: Growth Regulatory Properteis of Endothelins. *Peptides* 14,385-399, (1993)

51. Karsten Schror: Antiplatelt Drugs, a comparative review. *Drugs* 50(1),7-28 (1995)

52. D.L. Edward, C. P. Arora, D. T. Bui and L. C. Castro: Long-term nitric oxide blockade in the pregnant rat, Effects on blood pressure and plasma levels of endothelin-1. *Am J Obst G* 175(2), 484-488, (1996)

53. T. Kullmer, E. Jungmann, T. Haak and K. H. Usadel: Modification of the Responses of Endothelin-1 to Exhaustive Physical Exercise Under Simulated High-Altitude Conditions With Acute Hypoxia. *Metabolism* 448-9, (1995)

54. H. Nakamoto, Carlose M. Ferrario, Stanley B. Fuller, David L. Robaczewski, E. Winicove and Richard H. Dean: Angiotensin-(1-7) and Nitric Oxide Interaction in Renovascular Hypertension. *Hypertension* 25(2),796-802, (1995)

55. Y. Takeda, I. Miyamori,T. Yoneda and R. Takeda: Increased Concentration of Endothelin Messenger RNA in the Mesenteric Arteries of Cyclosporine-Induced Hypertensive Rats. *Am J Hypertens* 6,427-430, (1993)

56. Felix Bochner and V. John. Loyd: Aspirin for Myocardial Infarction; Clinical Pharmacokinetic Considerations. *Clin. Pharm* 28(6), 433-438, (1995)

57. W. Kiowski,L. Linder and Paul Erne: Vascular Effects of Endothelin-1 and Influence of Calcium Channel Blockade. *J Hypertens* 12 (Suppl 1)S21-S26, (1994)

58. T. Kuga, Y. Ohara,H. Shimokawa, S. Ibayashi, H. Tomopike and A. Takeshita: Inhibitory Effects of Aspirin on Coronary Hyperreactivity to Autocoids After Arterial Balloon Injury in Miniature Pigs. *J Cardio Ph* 25273-281, (1995)

59. G. W. Dorenn II. and M. W. Becker: Thromboxane $alpha_2$ Stimulated Signal Transduction in Vascular Smooth Muscle. *J Pharm Expt Ther* 265(1),447-456, (1993)

60. M. Hamberg, J. Svensson and B. Samuelsson: Thromboxane, A New Group of Biologically Active Compounds Derived from Prostaglandin Endoperoxidase. *Proc Natl. Acad. ci USA* 72,2994-2998, (1975)

61. S. Serebryakov,S. Zakharenko,V. Snetkov and K. Takeda: Effects of Prostagalndins E1 and E2 on Cultured Smooth Muscle Cellss and Strips of Aorta. *Prostagland* 4(47), 353-365, (1994)

62. A. Mebazaa,R. Wetzel,M. Cherian and M. Abraham: Comparison Between Endocardial and Great Vessel Endothelial Cells, Morphology, Growth, and Prostagalndin Release. *Am J Physiol 268 (Heart Circ Physiol* 37*)* H250-H259, (1995)

63. C. Kearn and J. Hirsch: Optimal Dose for Starting and Maitaining Low-Dose Aspirin. *Arch In Med* 153,700-702, (1993)

64. M. Lecomte,O. Laneuville,C. Ji, David L: DeWitt and Willima L. Smith. Acetylation of Human Prostaglandin Endoperoxidase Synthase-2 (Cyclooxygenase-2) by Aspirin. *J Biol Chem* 269(18), 13207-13215, (1994)

65. Lenard M. Lichtenberger, C. Ulloa, Amy L. Vanous, Jim J. Romero, El;izbeth J. Dial, Paul A. Illich and Edgart T Walters: Zwitterionic Phospholipids Enhance Aspirin's Therapeutic Activity, as Demonstrated in Rodent Model Systems. *J Phartm Exp Ther* 277(3),1221-1227, (1996)

66. Maria I. Roson, Mariana Maquiera-Kopmann, and Ignacio, J. de la Riva: Contrasting Effects of Norepinephrine and 5-Hydroxytryptamine on Contractility of Abdominal Aorta of Two Kidney Clip Hypertensive Rats. Effects of Inhibitors of Arachidonic Acid Metabolic Enzymes. *Clin Exp Hy-Theory and Practice* A12(2),285-306 (1990)

67. B. J. Smith and A. L. Willis: Aspirin selctively inhibits prostagalndin production in human platelets. *Nature* 231, 235-237, (1971)

68. J. R. Vane: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature* 231,232-235, (1971)

69. K. Kamata and A. Makino: A comparative study on the rat aorta and mesenteric arterial bed of the

possible role of nitric oxide in the desensitization of the vasoconstrictor response to an alpha$_1$-adrenoceptor agonist. *Br. J. Pharam* 120,1221-1228, (1997)

70. N. L. Kanagy,J. R. Charpie,J. Dananberg and C. Webb: Decreased sensitivity to vasoconstrictor in aortic rings after acute exposure to nitric oxide. *Am J. Physiol* 271, H253-H260, (1996)

71. V. Kalpakov,D. Gordon and T. J. Kulik: Nitric Oxide-Generating Compunds Inhibit Total Protein and Collagen Synthesis in Culture Vascular Smooth Muscle cells. *Cir Res* 76(2), 305-309, (1995)

72. R. Busse,A. Mulsch, I. Fleming and M. Hecker: Mechanism of Nitric Oxide Release From the Vascular Endothelium. *Circulation* 87(suppl V),V-18-V-25, (1993)

73. H. Moritoki,H. Miyano, S. Takeuchi,M. Yamaguchi,T. Hisayama. and W. Kondoh: Endothelin-3-Induced Relaxation of Rat Thoracic Aorta, A Role for Nitric Oxide Formation. *Br J Pharam* 108,1125-1130, (1993)

74. Gjurmrakch Alieve, Vera Ralelvic and Geoffrey Bursstock: Depression of Endothelial nitric Oxide Symthase but Increased Expression of Endothelin-1 Immunoreactivity in Rat Thoracic Aortic Endothelium Associatee With Long-term, but Not Short-term, Sympathectomy. *Circul Res* 79,317-323, (1996)
75. Nigel Benjamin and John Vane: Nitric Oxide and Hypertension. *Circulation* 94,1197-1198, (1996)

76. A. Giaid and D. Saleh: Reduced Expression od Endothelial Nitric Oxide Synthase in the Lungs of Patients with Pulmonary Hypertension. *N Engl J Med* 333,,214-221, (1995)

77. Anna F. Dominiczak and David F. Bohr: Nitric Oxide and Its Putative Role in Hypertension. *Hypertension* 25(6), 1202-1211, (1995)

78. U. Forstermann, E. I. Closs, J. Pollock, M. Nakane, P. Schwarz, I. Gath and H. Klein: Nitric oxide synthase isozymes: characterization, purification, molecular cloning and functions. *Hypertension* 23, 1121-1131, (1994)

79. W. M Xu, P. Gorman, D. Sheers, G. Bates, J. Kishimoto, L. Lizhi and P. Emson: Regional localization of the gene coding for human brain nitric oxide synthase (NOS) to 12q 24.2 -24.3 by flourescent in situ hybridization. *Cyto C Gen* 64, 62-63, (1993)

80. W. Xu, I. Charles, S. Moncada, O. Gorman, L. Liu and P. Emson: Chromosomal assignment of inducible NOS gene and endothelial NOS gene to human chromosome 17p11-17q11 and chromosome 7, respectively. *Endothelium (Supl)* S24, Abstract (1993)

81. P. A. Marsden, K. T. Schappert, H. S. Chen, M. Flowers, C. L Sundell, J. N. Wilcox S. Lames, T. Michael: Molecular cloning and characterization of human endothelial nitric oxide synthase. *FEBS Lett* 307, 287-293 (9192)

82. M. Tominaga, K. Fuji, I. Abe,Y. Takata,K. Kobayashi and M. Fujishima: Hypertension and Aging Impair Acetylcholine-Induced Vasodilation in Rats. *J Hypertens* 12,259-268, (1994)

83. L. Minowa, S. Miwa, S. Kobayashi, T. Enoki, X. F. Zhang, T. Komuro, Y. Iwamuro and T. Masaki: Inhibitory effect of nitrovasodialtors and cyclic GMP on ET-1-activated Ca^{2+}-permeable nonselective cation channel in rat aortic smooth muscle. *Br J Pharam* 120,1536-1544, (1997)

84. T. Weidelt, W. Boldt and F. Markwardt: Acetylccholone-induced K+ current in smooth muscle cells of intact rat small arteries. *J Physiol* 500(3),617-630, (1997)

85. G.J. Dusting, P.A. Dickens, R. D'Nicolantonio and A. E. Doyle: Vascular Prostacyclin and Goldblatt hypertensive rats. *Hypertension* 2,31-36 (1984)

86. A. Papapetropolulos, N. Marczin, Mary D. Snead, C. Cheng, A. Milici and John D. Catravas: Smooth Muscle Cell Responsiveness to Nitrovasodilators in Hypertensive and Normotensive. *Hypertension* 23,476-484, (1994)

87. J. Donickier, L. Stoleru, W. Hayashida, H. V. Mechelen, P. Selvais, L. Galanti, J. P. Clozel, J. M. Ketelslegers and H. P.Puoleur: Role of Endogenous Endothelin-1 in Experimental Renal Hypertension in Dogs. *Circulation* 92,106-113, (1995)

88. Chu C. Chua, Roland C. Hamdy, Balvin H. L. Chua: Regulation of endothelin-1 production by thromboxane A_2 mimetic in rat heart smooth muscle cells. *Biochim et Biophys Acta* 1313,1-5, (1996)

89. Guy H. Neild: Endothelin Plasma Levels in Hypertensive Patients with Vascular Disease. *J Hypertens* 12(1),S17-S20, (1994)

90. C. Peiro, J. Redpndo, M. A. Rodriguez-Martinez, J. Angulo, J. Marin and , Carlos F. Sanchez-Ferrer: Influence of Endothellium on Cultured Vascular Smooth Mescle Cell Proliferation. *Hypertension* 25(2),748-751, (1995)

91. A. Dellipizzi, M.L. Pucci, A. I. Mosny, K. Deseyn and A. Nasjeltti: Contribution of Constrictor Prostanoids to the Calcium Dependent Basal Tone in the Aorta from Rats with Aortic Coarctation-Induced Hypertension, Relationship to Nitric Oxide. *J Pharm Exp Ther* 283,75-81, (1997)

92. Birgitta, C.P. Huskin, Maarten, G.C. Hendriks, M. Pfaffendorf,and Pieter, A. van Zwieten: Effect of Aging and Hypertension on the Reactivity of Isolated Conduit and Resistance Vessels. *Microvasc R* 48, 303-315, (1994)

93. J. Torok and M. Gerova': Developmental dynamics of endothelial and neurogenic control of canine thoracic aorta. *Mech Age D* 95,143-152, (1997)

94. R. Rahimian, I Laher, G. Dube and C. van

Breemaen: Estrogen and Selective Estrogen Receptor Modulator LY117018 Enhances Release of Nitric Oxide in Rat Aorta. *J Pharm ExpTher* 283,116-122, (1997)

95. K. Kauser and G. B. Rubanyi: Gender Differences in Endothelial Dysfunction in the rat Aorta of Spontaneously Hypertensive Rats. *Hypertension* 25,517-523, (1995)

96. Mills, John A. Aspirin, The Ageless Remedy: *N Eng J Medi* 325(18),1303-1304, (1991)

97. F. Bochner, A. A. Somgyo and K. M. Wilson: Bioinequivalence of For 100mg Oral Aspirin Formulations in Healthy Volunteers. *Clin Pharmac* 21(5),394-399, (1991)

98. N. J. Davies, M. R. Gazvani, R. G. Farquharson and S. A. Walkinshaw: Low-Dose Aspirin in the Prevention of Hypertensive Disorders of Pregnancy in Relatively Low-Risk Nulliparous Women. *Hypertens P*14(1), 49-55 (1995)

99. John C. Hauth, Robert L. Goldenberg, Richard C. Parker, Jr., Joseph B.Philip, Rachel L. Copper, Mary B. DiBard and Gary R. Cutter: Low-dose aspirin therapy to prevent preeclampsia. *Am J Obst G*168,1083-1093, (1993)

100. T.F. Imperiale, , & A. Stollenwerk-Petrulis: A Meta-analysis of low dose aspirin for the prevention of pregnancy induced hypertensive disease. *JAm Med A* 266,261-265, (1991)

101. K.A. Louden, Fiona A. Broughton-Pipkin, E. M. Symond, P. Tuohy, C. O'Callahan, S. Heptinstall, S. Fox and J. R. A. Mitchell: A Randomized placebo-controlled study of the effect of low dose aspirin on platelt reactivity and serum thromboxane B2 production in non-pregnant women, in normal pregnancy, and in gestational hypertension. *Brit J Obst G* 99,371-376, (1992)

102. P. McParland, J. M. Pearce, & G. V. P. Chamberlain: Doppler ultrasound and aspirin in recognition and prevention of pregnancy-induced hypertension. *Lancet* 335,1552-1555, (1990)

103. F. Parazzini, C. Benedetto, T. Frusca, G. Gregorini, L. Bocciolone, L. Marozio and M. Romero: Low-dose aspirin in prevention and treatment of intrauterine growth retardation and pregnancy-induced hypertension. *Lancet* 341,396-400, (1993)

104. E. Schiff, E. Peleg, M. Goldenberg, T. Rosenthal, E. Ruppin, M. Tamarkin, G. Barkai, G. Ben-Baruch, I. Yahal, J. Blankenstein, B. Goldman & S. Mashiach: The use of aspirin to prevent pregnancy-induced hypertension and lower the ratio of thromboxane A2 to prostacyclin in relatively high risk pregnancies. *N Eng J Med* 321,251-356, (1989)

105. S. Uzan, M. Beaufils, G. Breart, B. Bazin, C. Capitant and J. Paris: Prevention of fetal growth retardation with low-dose aspirin, findings of the EPREDA trial. *Lancet* 337(8755)1427-1431, (1991)

106. H. C. S. Wallenberg, G. A. Dekker, J. W. Makovitz & P. Rotmans: Low-dose aspirin prevents pregnancy-induced hypertension and preeclampsia in angiotensin-sensitive primigravidae. *Lancet* 1,1-3, (1986)

107. Rober S. Adelstein and James R. Sellers: Effects of Calcium on Vascular Smooth Muscle Contraction. *Am J Card* 59, 4B-10B (1987)

108. C. Bloomstrand, J-E. Olsson,B. Nilsson, M. von Arbin, M. Britton, C-E. Elwin, C. Helmers, B. Norrving, A. Rosen, K.Samuelsson, K. Strandberg and N. G. Wahlgren: Swedish Aspirin Low-dose Trial (SALT) of 75 mg aspirin as secondary prophjylaxis after cerebrovascular ischemic event. *Lancet* 338,1345-1349, (1991)

109. M. Buerk, W. Pittroff, J. Meyer and H. Darius: Aspirin therapy, Optimized platelet inhibition with different loading and maintenance doses. *Am. Heart J* 130,465-472, (1995)

110. C. Cimminiello and M. Milani: Diabetes mellitus and peripheral vascular disease, is aspirin effective in preventing vacular events? *Diabetolog* 39,1402-1404, (1996)

111. Nananda F. Col, J. Yarzebski, Joel M. GoreJoseph S. Alpert and Robert J. Goldberg: Does Aspirin Consumption Affect the Presentation or Severity of Acute Myocardial Infarction? *Arch In Med* 155,1386-1389, (1995)

112. J.R. Copeland, K.A. Willoughby, T. M. Tynana, S. F. Moore and E. F. Ellis: Endothelial and non endothelial cycooxygenase medaite rabbit pial arteriole dialtion by bradykinin. *Am J Physiol 268 (Heart Circ. Physiol 37)*,H458-466, (1995)

113. Garret A. Fitzgerald, Mary Lupinetti, Susan A. Charman and Willima N. Charman: Presystemic Acetylation of Platelets by Aspirin, Reduction in Rate of Drug Delivery to Improve Biochemical Selectiveity for Thromboxane A2. *J Pharm Exp Ther* 259 (3), 1043-1049, 1991.

114. J. S. Floris: Epinephrine and Genesis of Hypertension, *Hypertension* 19,1-18, (1992)

115. Thomas Force, Richard Milani, Patricia Hibberd, Reinhard Lorenzw, Waltraud Udelhoven, Alexander Leaf and Petere Weber: Aspirin-Induced Decline in Prostacyclin Production in Patients With Coronary Artery Disease Is Due to Decreased Endoperoxide Shift. *Circulation* 84,2296-2293, (1991)

116. Jan, V. Gijin, A. Algra, J. Kappelle and A. van Latum: A comparison of the Two doses of Aspirin (30 mg vs. 283 mg A Day) in patients after a transient Ischemic Attack or Minor Ischemic Stroke. *N Eng J Med* 325(18),1261-1266, (1991)

117. Allen W. Gomoll and Martin L. Ogletree: Failure of aspirin to interfere with the cardioprotective effects of ifetroban. *Eur J Pharm* 271,471-479, (1994)

118. S.E., Hankinson, J. M., Seddon, G. A. Colditz, M. J. Stampfer, B. Rosner, F. E. Speizer and W. C. Willet: A Prospective study of Aspirin Use and Cataract Extraction in Women. *Arc Ophth* 111,503-508, (1993)

119. J. M. Herbert, A. Bernat, M. Samama and J. P. Maffrand: The Antiaggregating and Antithrombotic Activity of Ticlopidine Is Potentiated by Aspirin in the Rat. *Thromb Haem* 76(1),94-98, (1996)

120. T. W. Meade and G. J. Miller: Combined Use of Aspirin and Warfarin in Primary Prevention of Ischemic Hear Disease in Men at High Risk. *Am J Card* 75,23B-26B, (1995)

121. S. Rotondo, D. Rotilio, C. Cerletti and G. de Gaetano: Red Wine, Aspirin and Platelet Function. *Thromb Haem* 72(1),813-819, (1994)

122. Roger G. Thomas, H. Thibadeaux, Carol J. Erret, Martin M. Bednar, Cordell E. Gross and Wiliam F. Bennett: Intravenous Aspirin Causes a Paradoxical Attenuation of Cerebrovascular Thrombolysis. *Stroke* 26,1039-1046 (1995)

123. J. R. Vane and R. M. Botting. Heart Disease, Aspirin, and Fish Oil: *Circulation* 84(6), 2588-2590, (1991)

124. Lars C Wallentin: Aspirin (75 mg/day) After an Episode of Unstable Coronary Artery Disease, Long-Term Effects on the Risk for Myocardial Infarction, Occurence of Severee Angina and The Need for Revascularization. *J Am Col C* 18,1587-1593, (1991)

125. C. Watala and K. Gwozdzinski: Effect of Aspirin on Confirmation and Dynamics of Membrane Proteins in Platelets and Erythrocytes. *Biochem Pharm* 45(6),1343-1349, (1993)

126. Z. Dong, C. Huang, R. E. Brown and W.Y. Ma: Inhibition of Activator Protein 1 Activity and Neoplastic Transformation by Aspirin. *J Biol Chem* 272(15),9962-9970, (1997)

127. Michael J. Thun, Mohan M. Namboodiri and Clark W. Heath, Jr.: Aspirin Use and Reduced Risk of Fatal Colon Cancer. *N Eng J Med* 325 (23),1593-1596, (1991)

128. E. Kopp, and S. Ghosh: Inhibition of NF-kB by Sodium Salicylate and Aspirin. *Science* 265956-959, (1994)

129. J. P. Bourreau, Z.D. Zhang, A.M. Low, C.Y. Kwan and E.E. Daniel: Ryanodine and Adrenergic, Purinergic Stimulation in the Rat Vas Deferens Smooth Muscle, Functional and Radioligand Binding Studies. *J Pharm Exp Ther* 256(3), 1063-1071 (1991)

130. Methias W. Reipe, K. Kasischke and Anett Raupach: Acetylsalicylic Acid Increases Tolerance Against Hypoxix and Chemical Hypoxia. *Stroke* 28,2006-2011, (1997)

131. R. Hamid, M. Robinson and J. M. Pearce: Low dose aspirin in women with raised maternal serum alpha-fetoprotrein and abnormal Doppler waveform pattern from the uteroplacental circulation. *Brit J Obst G* 101, 481-484 (1994)

132. L. M. Lichtenberger: The hydrophobic barrier properties of gasterointestinal mucuc. *Ann R Physl* 57,565-583, (1995)

133. L. M. Lichtenberger, Z. M. Wang, M, N. Giraud, J. J. Romero and J. C. Barreto: Effect of naproxen on gastric mucosal hydrophobicity (Abstract) *Gastroenty* 108, A149, (1995)

134. L. M. Lichtenberger, Z. M. Wang, J. J. Romero, C. Ulloa, J. C. Perez, M, N. Giraud and J. C. Barreto: Non-steroidal and anti-inflamatory drugs (NSAID) associate with zwitterionic phospholipids, Insight into the mechanisms and reversal of NSAID-induced gastrointestinal injury. *Nature Medicine* 1, 154-158, (1995)

135. B. Ali and . Kaur: Mammalian tissue acetylsalicylic esterase(s), identification, distribution and discrimination from other esterases. *J Pharm Exp Ther* 226,594-598, 1983.

136. Marco, A. C. Benedito: Flourimetric Determination of Tissue Distribution and Differences Between the Activity of Aspirin Esterases I and II in Mice and Rats. *J Pharm Pha* 49,273-276, (1997)

137. P. A. Harris and S. Riegelman: Acetylsalicylic acid hydrolysis in human blood and plasma. *J Pharm Sci* 56,713-716, (1967)

138. L.-A. Pini, G. Vitale and M. Sandrini: Serotonine and Opiate involvement in the Antinccociceptive Effecti of Acetylsalicylic Acid. *Pharmacol* 54,84-91, (19967)

139. S.H. Ferreira, S. Moncada and J. R. Vane: Prostagalndins and the mechanism of analgesia produced by aspirin-like drugs. *Br J Pharm* 49,86-97, (1973)

140. Clark W. Heath, Michael J. Thun, E. R. Greenberg, B . Levin and Lawrence J. Marnett: Nonsteroidal Antiinflammatory Drugs and Human Cancer. *Cancer* 74,2885-2888, (1994)

141. M. L. Klein: Macular Degneration, Is Aspirin a Risk for Progressive Disease? *j Am Med A* 266(16)2279, (1991)

142. D. Conte, M. Nordio, S., Fillo, G. deGiorgio, A. Isidori and F. Romanelli : Aspirin Inhibition of Naloxone-Induced Luteinizing Hormone Secretion in Man. *J Clin End* 81(5),1772-1775, (1996)

143. F. Y. Li, Diane H. Russel, Stuart I. MyersNorman W. Weisbrodt and Frank G. Moody: Gallbladder Contrctility in Aspirin- and Cholestrol- Fed Prairie Dogs. *Gastroent y* 106, 1662-1667, (1994)

144. John V. Bosso, Lawrence B. Schwartz and Donald D. Stevenson: Tryptase and histamine release during aspirin-induced respiratory reaction. *J Allerg Cl*

88,830-837, (1991)

145. Neil H. Graham, Christopher J. Burrell, Robert M. Douglas, P. Debelle and L. Davis: Adverse Effects of Aspirin, Acetominophen, and Ibuprofen on Immune Function, Viral Shedding, and Clinical Status in Rhinovirus-Infected Volunteers. *J Infec Dis* 162,1277-1282, (1990)

146. R. E. Shakelford, P. B. Alford, Y. Xue, S.F. Thai, D. O. Adams and S. Pizzo: Aspirin Inhibits Tumor Necrosis Factor-z Gene Expression in Murine Tissue Macrophages. *Molec Pharm* 52,421-429, (1997)

147. W. R. WIlliams and A. Pawlowicz: Aspirin-Sensitive Asthma, Significance of the Cyclooxygenasae-Inhibiting and Protein-Binding Properties of Analgesic Drugs. *Int A Al Im* 95,303-308, (1991)

148. S. O'Sullivon, B. Dahlen, Sven E Dahlen and M. Kumlin: Increased urinary excretion of the prostagalndin D2metabolite 9a, 11b-prostaglandin F2 after aspirin challenge supports mast cell activation in aspirin- induced airway obstruction. *J Allerg Cl* 98,421-432, (1996)

149. B. McAdam, R. M. Keimowitz, M. Maher and D. J. Fitzgerald: Transdermal Modification of Platelt Function, An Aspirin Patch System Results in Marked Suspension of Platlet Cucooxygenase. *J Pharm Exp Ther* 277(2),559-564, (1996)

150. W. D. Steyn and H. Odendaal: Randomised controlled trial of ketanserin ans aspirin in prevention of pre-eclampsia. *Lenacet* 350,1267-1271, (1997)

151. G. Kwon, J. R. Hill, J. A Corbet. and M. L. McDaniel: Effects of Aspirin on Nitric Oxide Formation and De Novo Protein Synthesis by RINm5F Cells and Rat Islets. *MolecPharm* 52398-405, (1997)

152. Y. Merhi, J. Bernier, Y. Marois and R. Guidoin: Acute Thrombogenicity of Arterial Prostheses Exposed to Reduced Blood Flow in Dogs, Effects of Heparin, Aspirin, and Prostacyclin. *J Cardio Ph* 26,1-5, (1995)

153. R. R. Taylor, F. A. Gibbons, G. D. Cope, G. N. Cumpston, G. C. Mews and P. Luke: Effects of Low-Dose Aspirin After Cornary Angioplasty. *Am J Card* 68,874-878, (1991)

154. P. T. Larsson, N. H. Wallen and P. Hjemdahl: Norepinephrine-Induced Human Platelet Activiation iIn Vivio Is Only Partly Counteracted by Aspirin. *Circulation* 891951-1957, (1994)

155. E. J. Willard, R. A. Lange and D. L. Hillis: The Use of Aspirin in Ischemic Heart Disease. *N Eng J Medi* 327(3), 175-181, (1992)

156. Victoria L. Lamb, Annette J. Schwartz, William R. Rohn and Lana. Kaiser: Cyclooxygenase Inhibitors Depress Norepinephrine Constriction of Rat Abdominal, But Not Thoracic , Aorta. *Eur J Pharm* 256221-226, (1994)

157. M. A. Rahmani, T. Mangroo, M. Neves, M. Bienaime, S. Wiggins. and J. Williams: Effects of Aspirin on the Contractility of Aortic Rings in vitro from Spontaneously Hypertensive Rats. *Artery* 20(3),135-146, (1993)

158. M. A. Rahmani, T. Mangroo, M. Bienaime, S. Wiggins and J. Williams: Effect of Aspirin on the Contractility of Aortic Smooth Muscle in Female Spontaneously Hypertensive Rats. *Artery* 19 (5),271-283, (1992)

159. J. L. Mehta, P. Mehta and D. Lawson: Alterations in Platelet Alpha 2-Adrenoceptors by Aspirin. *Agent Actio* 24,196-203, (1988)

160. M. Grilli, M. Pizzi, M. Memo and Pier F. Spano: Neuroprotection by Aspirin and Sodium Salicylate Through Blockade of NF-kB Activation. *Science* 274,1383-1385, (1996)

161. K. Yin, Z. M. Chu and L. J. Beilin: Effect of fish oil feeding on blood pressure and Vascular Reactivity in Spontaneously Hypertensive Rats. *Clin Exp Ph* 17,235-239, (1990)

162. M. A. Rahmani, V. David,M. Huang and G. DeGray: Effect of Aspirin on the Contractility of Aortic Smooth Muscle and the Course of Blood Pressure Development in Male Spontaneously Hypertensive Rats. Accepted for *Artery* 22(6) or 23(1) (1998)

163. K. Nishimura, Ota Mikio and Ito Katsuaki: Existence of two components in the tonic contraction of rat aorta mediated by alpha$_1$-adrenergic activation. *Br J Pharm* 102,215-221 (1991)

164. P. B. Furspan, G. J. Rinaldi, K. Hoffman and D. F. Bohr: Dietary Calcium and Cell Membrane Abnormalities in Genetic Hypertension. *Hypertension* 13(6) Part 2, 727-730, (1989)

165. David A. McCarron: Is Calcium More Important Than Sodium in the Pathogenesis of Essential Hypertension? *Hypertension*. 7(4), 607-613, (1985)

166. N. Decker, J. D. Ehrhardt, G. Leclerc and J. chawrtz: Post Junctional a-Adrenoceptors, alpha$_1$- and alpha$_2$- Subtypes in Rat Vas culature in vitro and *in vivo*. *Nuauyn-Schmiedbergs Arc Pharmacol* 326,1-6, (1984)

167. M. A. Rahmani, I. R. Cheema, S. Sen and B. Peoples: Evaluation of Isomers of Octopamine for In Vitro alpha- Adrenergic Stimulation of the Aortic Smooth Muscle from Spontaneously Hypertensive Rats. *Cytobios* 52,7-16, (1987)

168. M. A. Rahmani, I. R. Cheema, S. Sen, B. Peoples and S. Riley, S: Effect of Hypothyroidism on alpha$_1$- and alpha$_2$-Adrenergic Responsiveness in Rat Aortic Smooth Muscle. *Artery* 14(6), 362-383 (1987)

169. M. A. Rahmani, M., Neves, T. Mangroo. and T. Bennett: In Vitro Caffeine Induced Aortic Smooth Muscle Reactivity In Rat. *Artery* 17 (3),127-143

(1990)

170. Theophile Godfraind: Classification of Calcium Antagonists. *Am J Card* 59,11B-23B, (1987)

171. Imad A. Alhaddad, L. Tkaczevski, F. Siddiqui, R. Mir and Edward J. Brown Jr: Aspirin Enhances the Benefits of Late Reperfusion om Infarct Shape. *Circulation* 91,2819-2823, 1995

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