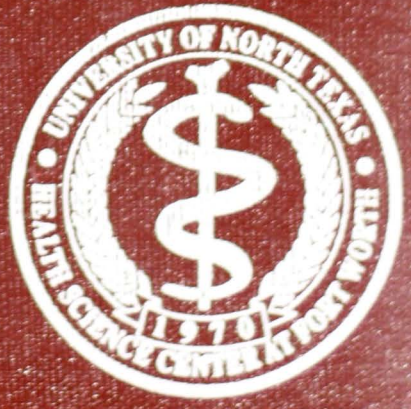
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This study was designed to determine the effects of renovascular hypertension (RVH) on coronary vasoreactivity in conscious, chronically instrumented dogs. Six dogs were instrumented to measure left ventricular pressure, $+dP/dt_{max}$, heart rate, mean aortic pressure, circumflex blood flow (CBF), and cardiac output. In order to examine endothelial-dependent and independent coronary vasodilation, intracoronary injections of acetylcholine (ACh), bradykinin (BDK), and sodium nitroprusside (SNP) were studied before and after induction of RVH in the presence and absence of nitric oxide (NO) blockade. After RVH, resting CBF was significantly reduced ($P<0.05$). In the normotensive state, NO-blockade significantly reduced the coronary vasodilation to ACh and BDK ($P<0.05$), but not SNP. After RVH, the coronary vasodilation to ACh, BDK, and SNP were reduced ($P<0.05$). After RVH, NO-blockade further reduced the coronary vasodilation to BDK ($P<0.05$), but not ACh. Thus, RVH resulted in an impairment of both endothelial-dependent and –independent coronary vasodilation. It also appears that during RVH the endothelium retains the ability to produce/release NO to some, but not all, stimuli. In order to examine the possibility that β-adrenergic mediated coronary vasodilation is impaired after RVH, intracoronary injections of norepinephrine (NE), isoproterenol (ISO), and terbutaline (TRB) were administered. These drugs all caused dose dependent increases in CBF before and after RVH. After RVH, the coronary

vasodilatory responses to NE, ISO and TRB were significantly reduced ($P<0.05$). β_1-blockade with intracoronary atenolol (1 mg) reduced the ISO-induced increases in CBF and had no effect on TRB responses ($P<0.05$). β_2-blockade with intracoronary ICI-118,551 (1 mg) reduced the ISO-induced coronary vasodilation and abolished TRB responses ($P<0.05$). During β_2-blockade, ISO-induced increases in CBF were not different after RVH. Therefore, these data indicate that β_1-adrenergic mediated coronary vasodilation is preserved after RVH, whereas, β_2-mediated is not. We conclude that 1) RVH results in an impairment of both endothelial-dependent and –independent coronary vasodilation; 2) RVH results in an impairment of β_2-adrenergic mediated coronary vasodilation.

DYSFUNCTIONAL CONTROL OF CORONARY BLOOD FLOW IN

RENOVASCULAR HYPERTENSION

Geoffrey P. Kline, B.S.

APPROVED:

Patricia A. Gwirtz
Major Professor

Barbara L. Baron
Committee Member

Committee Member

Committee Member

Committee Member

Committee Member

Chair, Department of Integrative Physiology

Dean, Graduate School of Biomedical Science

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Geoffrey P. Kline, B.S.

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CHAPTER I

INTRODUCTION AND BACKGROUND

Regulation of blood flow to the working myocardium involves an extraordinarily complex system of interacting mediators. In the simplest terms, coronary blood flow is directly proportional to the arterial-venous pressure gradient across the heart and inversely proportional to the resistance to flow in the vasculature. Coronary vascular resistance is modulated by mechanical, neural, metabolic, myogenic, humoral, and endothelial mechanisms.

Coronary resistance in the contracting left ventricle is influenced by the mechanical contraction of surrounding tissue during systole, known as extravascular systolic compression.[1] The remaining modulators of coronary resistance (neural, metabolic, myogenic, humoral, and endothelial) interact to ultimately control vascular smooth muscle tone.[1,2] The arterial circulation of the heart is composed of diverse groups of vessels including conductance arteries, small arteries, and arterioles, each of which contains unique functional differences. For example, large epicardial vessels contribute only 2-5% of total coronary resistance and small arteries account for approximately 20-35% of total coronary resistance; however, the majority of resistance (>50%) is imposed by the downstream arterioles.[3,4] This characteristic is mainly attributable to the anatomical size and number of arterioles relative to the large and small

arteries. It is well documented in the literature that these different populations of vessels also contain many differences in physiological and pharmacological characteristics.[3-5]

Neural Control

Parasympathetic Control

Neural control of coronary blood flow involves innervation by both the parasympathetic and sympathetic branches of the autonomic nervous system. The existence of parasympathetic (cholinergic) innervation of both large and small coronary arteries has been established using acetylcholinesterase staining. In the large coronary arteries, parasympathetic innervation terminates at the adventitial-media border.[6] In the small arteries parasympathetic innervation extends to the outer medial layer.[7] The neurotransmitter, acetylcholine, is released from these nerves upon stimulation, which causes vasodilation in the coronary arteries. Exogenous administration of acetylcholine has been termed endothelium-dependent vasodilation because in the absence of an intact endothelium, acetylcholine causes vasoconstriction mediated by smooth muscle cholinergic muscarinic receptor activation.[8] It is now well established that exogenously applied acetylcholine results in the production of endothelium-derived relaxing factor (EDRF), believed to be nitric oxide (NO). Some controversy exists, though, on the ability of physiological parasympathetic stimulation to elicit coronary vasodilation. Intraluminally applied exogenous acetylcholine directly interacts with the endothelium causing vasodilation. However, acetylcholine released from parasympathetic nerves must first diffuse through the medial layer of the artery before reaching the endothelium.

Studies have demonstrated that extraluminally applied acetylcholine has the ability to diffuse through the vascular wall and cause endothelium-dependent vasodilation.[9,10] Broten *et al.*[11] reported that coronary vasodilation caused by both intrarterial infusion of acetylcholine and by vagal stimulation were similarly reduced by inhibition of NO synthesis. They concluded that parasympathetic coronary vasodilation is dependent on NO/EDRF production. It is still unclear how the parasympathetic nervous system contributes to the physiological control of coronary blood flow in normal as well as pathological conditions.

Sympathetic Control

All segments of the coronary microvascular tree receive sympathetic innervation, although the density of innervation appears to be different at various vascular levels.[2] Large arteries carry a plexus of nerves where several fibers innervate a single vessel, while small arterioles generally have a bipolar distribution of (2) sympathetic fibers.[12] Stimulation of these sympathetic nerves results in release of norepinephrine accompanied by various cotransmitters such as neuropeptide Y and enkephalins.[2,13] These neurotransmitters then act on a myriad of both vascular and myocardial receptors. One of the most studied classes of receptors in the coronary circulation is the α-adrenergic receptor. Despite the greater sympathetic innervation of arteries than of arterioles, activation of α-adrenergic receptors with either norepinephrine or sympathetic

stimulation results in similar increases in arterial and arteriolar resistances.[3] This may be due to the differences in distribution of receptors in the coronary circulation.

The α_1-receptor is generally thought to be located postsynaptically on vascular smooth muscle, where it causes vasoconstriction.[14] The α_2-receptor is classically thought to be located only on the presynaptic nerve ending, where it provides negative feedback to limit norepinephrine release.[15] However, recent studies have challenged these concepts. Evidence has been presented for the existence of presynaptic α_1-receptors as well as postsynaptic α_2-receptors.[16,17] Postsynaptic α_2-receptors have been demonstrated on both the vascular smooth muscle, where they cause vasoconstriction, as well as on the endothelium, where they elicit endothelium-dependent NO-induced vasodilation.[18-20].

Based on functional studies, it appears that the distribution of the α-adrenoceptor subtypes is heterogeneous throughout the coronary circulation. The α_1-adrenoceptors may be homogeneously distributed throughout the coronary microcirculation, whereas the α_2-adrenoceptors are primarily located in arterioles less than 100 μm in diameter.[5,21,22] Evidence for this has been observed in studies of the canine heart which demonstrate that α_1-adrenergic constriction occurs mainly in small coronary arteries greater than 100 μm in diameter, while α_2-adrenergic constriction predominantly occurs in the coronary arterioles less than 100 μm in diameter.[4,5,23] These findings suggest a similarity to skeletal muscle microcirculation in the distribution of α_1- and α_2-adrenoceptors.[23]

Norepinephrine released as a result of sympathetic stimulation simultaneously acts on another important class of adrenergic receptors, the β-receptors, in the coronary circulation. Stimulation of coronary β-receptors cause vasodilatation through a variety of mechanisms. The coronary circulation contains both β_1- and β_2- adrenergic receptors. The distribution of these receptors is heterogeneous, much like that of the α-adrenoceptors. Autoradiographic and radioligand binding studies support the hypothesis that β_1- and β_2-receptors in the coronary vascular tree are distributed differently.[24,25] Studies with autoradiography have shown that the density of β-receptors is inversely related to coronary vessel diameter.[24] Thus, canine arterioles (20 – 70 μm in diameter) have a β-receptor population ratio of approximately 10% β_1 to 90% β_2.[25] However, large canine epicardial arteries have a ratio of 85% β_1 to 15% β_2.[26] These studies are in agreement with the results of radioligand-binding studies of large epicardial vessel membrane homogenates that found a greater proportion of β_1-receptors than β_2-receptors.[27]

The β-adrenoceptors are known to exist on several different cell types in the coronary microcirculation. The myocardium contains both β_1- and β_2- receptors; stimulation of these receptors results in increased heart rate and contractility.[28,29] This leads secondarily to a local metabolic feedback vasodilation.[2] Radioligand-binding studies performed on smooth muscle of bovine large coronary arteries have demonstrated the presence of both β_1- and β_2-adrenergic receptor subtypes.[30] There is growing evidence of the existence of β_2-receptors located on the endothelium.[31-33] Several

studies have shown that β_2-receptor mediated vasodilation in coronary resistance vessels is dependent on endothelial NO formation.[31,32] However, in large coronary conductance vessels, β_2-adrenergic receptors do not appear to be functionally coupled with NO synthesis. Rather, it is thought that β_2-receptor stimulation results in large vessel dilation that is largely a flow-dependent phenomenon.[33,34] It is important to consider the fact that in the large coronary conductance vessels β_1-receptors predominate, which are not coupled to endothelial-NO production; however, in the small resistance vessels the β_2-receptors predominate, where they appear to be coupled to NO production.

Physiological increases in myocardial oxygen consumption result from sympathetic nervous system release of norepinephrine. The resulting vasodilation is a result of a balance between the effects of stimulation of α- and β-adrenoceptors. Norepinephrine stimulation of myocardial β_1-receptors results in increased myocardial oxygen consumption, which then creates local metabolic feedback vasodilation.[2] The imbalance between metabolic demand and oxygen delivery provides an error signal for the metabolic feedback system to operate and cause local vasodilation.[2] Miyashiro and Feigl reported has also found evidence for a direct feedforward β-receptor mediated coronary vasodilation as well, which was independent of chronotropic and inotropic effects.[35] Since feedforward systems do not require an error signal, this feedforward vasodilation could allow a rapid increase in oxygen supply while minimizing the imbalance between supply and demand, thereby decreasing the error signal generated.[35] Therefore, the combined effects of a closed-loop negative feedback and open-loop

feedforward control mechanisms would allow for the rapid matching of coronary blood flow and myocardial oxygen consumption.

In summary, sympathetic neural control of the coronary circulation involves activation of both α- and β-adrenoceptors to provide increases in myocardial oxygen consumption, indirect metabolic vasodilation, direct vasodilation, and vasoconstriction. It is apparent that the differences in receptor population along the coronary vascular tree allow for this integrated effect.

Metabolic and Myogenic Control

Autoregulation is the ability to maintain blood flow constant at varying intraluminal pressures. In the coronary microcirculation, autoregulation is primarily a function of adjustments mediated by myogenic and metabolic mechanisms.[2] Both of these mechanisms play an important role in modulating coronary blood flow; however, the relative significance of each varies in different segments of the microcirculation.[36]

Myogenic control is based on the intrinsic property of the blood vessels, specifically the vascular smooth muscle, to regulate vascular tone in response to changing intraluminal pressure. In general, increases in intraluminal pressure elicit vasoconstriction, whereas decreases in pressure elicit vasodilation. Myogenic mechanisms, however, likely play a varying role in autoregulation throughout the microcirculation. Small coronary arteries, 150 –300 μm in diameter, respond passively to changes in transmural pressure.[36] This implies a lack of myogenic activity. However, it has been demonstrated that isolated coronary arterioles, 40-100 μm in diameter, display

significant myogenic activity.[37] Myogenic activity is not altered by removal of the endothelium, indicating that it is an intrinsic property of the vascular smooth muscle.

Myogenic coronary regulation also varies according to transmural differences. It has been observed that the subepicardial coronary arterioles are more myogenically active than those of the subendocardium.[37] This applies to responses to both high and low transmural pressures.

The local metabolic control of the coronary circulation is thought to be one of the most significant mechanisms of controlling coronary blood flow.[2] The working myocardium produces and releases of various metabolites, including adenosine, adenine nucleotides (ATP and ADP), hydrogen ions (H^+), and potassium ions (K^+). As the metabolic activity increases, so does the release of these metabolites. These compounds decrease vascular resistance and increase coronary flow to meet the myocardial oxygen/nutrient demand.[2,38] However, the exact metabolite or metabolites responsible for this hyperemia have not been clearly identified.[36] It has been shown, though, that autoregulation and the reactive hyperemic response to an occlusion can be completely inhibited by glibenclamide, a ATP-sensitive potassium channel antagonist.[39,40] Yet, it is unclear what mechanism(s) are responsible for the activation of these smooth muscle membrane channels.

Humoral Control

Numerous circulating humoral substances also play various, and often undetermined, roles in regulating coronary blood flow. Some of these include:

norepinephrine, epinephrine, vasopressin, angiotensin I and II, neuropeptide Y (NPY), enkephalins, renin, bradykinin, calcitonin-gene-related peptide (CGRP), substance P, atrial natriuretic peptide (ANP), and brain natriuretic peptide (BNP). Angiotensin II is supplied to the coronary vessels by spillover from the systemic circulation as well as locally generated angiotensin II via a local coronary renin-angiotensin-system.[41] Angiotensin II causes significant vasoconstriction and enhances sympathetic drive by presynaptic facilitation of norepinephrine release.[42] Angiotensin II also has the ability to potentiate the constrictor response to endothelin, a potent endothelium-produced vasoconstrictor.[42] Bradykinin is formed from kininogen in both the plasma and the endothelial lining; it causes vasodilation by stimulating production and release of endothelial-NO. The catecholamines cause both vasodilation (via β-receptors) and vasoconstriction (primarily via α-receptors).[1,2,43]

ANP and BNP are structurally and functionally related. It appears that they both cause vasodilation by stimulating vascular smooth muscle guanylyl cyclase, resulting in an increase in cGMP.[42,44] This action is similar to that of the nitrovasodilators. However, it is unknown whether these compounds, along with many of the others mentioned above, have a physiological or pathological role in regulating coronary vascular tone.

Endothelial Control

In addition to the previously mentioned regulatory mechanisms, vascular endothelial cells play a major role in modulating coronary blood flow. This control is

manifested by endothelial release and metabolism of, and/or interactions with, various substances including: nitric oxide (NO), prostacyclin (PGI_2), adenosine, endothelium-derived hyperpolarizing factor (EDHF), and endothelins (ET's).[45-47] Synthesis of these factors can be induced by various different pathways involving activation of endothelial purinergic, muscarinic, adrenergic, and kinin receptors; shear forces, ischemia, and hypoxia. This allows the endothelial lining of blood vessels to play a crucial paracrine role in regulating vascular smooth muscle function.

Nitric Oxide

The interactions between endothelial cells and the underlying vascular smooth muscle were first revealed in 1980 by Furchgott *et al* in their discovery that acetylcholine-induced vasodilation was dependent on the presence of vascular endothelium.[8] Since that discovery, research has described the mechanism responsible for this vasodilation as a release of a generically termed endothelium-derived relaxing factor (EDRF). It is now accepted that this EDRF is, in fact, chiefly nitric oxide (NO), or some very similar compound.[48] In addition to acetylcholine, many other substances, have since been shown to cause endothelial-NO-dependent vasodilation; these will be discussed later.

Undoubtedly, one of the most studied endothelial-dependent vasodilator mechanisms is that of the synthesis and release of NO. In the normal endothelium, NO is continuously synthesized and released, but not stored. NO is synthesized from L-arginine by a constitutive enzyme, termed endothelial-nitric oxide synthase (eNOS). This

reaction requires the presence of several cofactors such as NADPH, tetrahydrobiopterin, flavin adenine dinucleotide (FAD), and flavin adenine mononucleotide (FMN). [42] The enzyme eNOS is tightly regulated by Ca^{2+}-calmodulin and is therefore, stimulated by Ca^{2+} influx.[42]

The NO radical has a brief existence, having a half-life of approximately 5-8 seconds. During this time the NO can diffuse intraluminally, where it inhibits leukocyte and platelet adhesion, or abluminally, toward the vascular smooth muscle, where it modulates vascular tone and growth.[42] Once the NO reaches and diffuses into the vascular smooth muscle cells, it activates soluble guanylate cyclase. This results in an increase in the second messenger cGMP, leading to cGMP-dependent relaxation. NO has been hypothesized to also have several cGMP-independent effects on vascular smooth muscle[49]. These cGMP-independent effects of NO possibly include inhibition of protein expression, stimulation of prostaglandin production, depression of G-protein and of Ca^{2+} activity, elevation of K^+ channel activity, inhibition of gap junctions and cellular proliferation[49]. Thus, it is likely that NO has actions in the vasculature that involve both cGMP-dependent and cGMP-independent mechanisms. It is hypothesized that acute stimulation of endothelial-derived NO, above basal levels, leads to vasodilation, whereas, chronically elevated NO levels may lead to a generalized dampening of vascular reactivity[49].

NO has also been shown to cause hyperpolarization of vascular smooth muscle, which might then further add to the relaxation response by closing voltage-dependent

Ca^{2+} channels.[50] NO is principally inactivated by superoxide anions produced by various free-radical-generating mechanisms.[51,52] Superoxide anions are capable of inactivating both extracellular and intracellular NO.[51]

The importance of basal NO release in maintaining normal systemic blood pressure has been thoroughly demonstrated in numerous human and animal studies.[52,53] However, evidence of a role of NO release in maintaining coronary vasomotor tone is equivocal. In some human studies NO release has been found to be an important factor in regulating basal vasomotor tone.[52,54] Yet, observations in our laboratory and in the literature have shown that, in the canine, blockade of coronary endothelial-NO production has no effect on resting coronary blood flow.[31,32,46,55,56] Therefore, in the canine model, basal NO production and release do not appear important in maintaining resting coronary blood flow. It is however possible, that NO does in fact play a role in regulating basal flow, and once removed another mechanism takes over, resulting in normal blood flow. In support of this hypothesis, Jones *et al* found that in anesthetized dogs, inhibition of NO production with an L-arginine analog resulted in no change in coronary blood flow.[55] However, they noted that small coronary arteries constricted after NO inhibition, while arterioles dilated. They reasoned that basal, probably flow-dependent, NO release normally reduces vascular tone in the larger vessels, whereas in the smaller arterioles it did not play a significant role. The dilation of these smaller arterioles, after NO inhibition, is most likely a function of an intrinsic autoregulatory response, possibly metabolic or myogenic in origin.

Shear stress, i.e. flow-dependent viscous drag physically transmitted to the endothelial layer, is one of the most significant non-receptor-mediated mechanisms to stimulate endothelial NO production.[36] The resultant dilation to increased flow is termed "flow-dependent dilation" and is an important component of coronary vascular regulation. Flow- dependent dilation is an integral part of many physiological stimuli, including functional and reactive hyperemias.[34,36,42] Although the exact mechanism(s) of signal transduction have not been fully elucidated, shear stress is thought to activate K^+ channels that cause hyperpolarization.[42,57] Hyperpolarization allows Ca^{2+} entry through leak channels, driven by the now greater electrochemical gradient. Thus, virtually any intervention or physiologic response that increases coronary blood flow (increased shear stress) will increase endothelial intracellular Ca^{2+}, stimulating NO production/release.

The coronary endothelium expresses a multitude of receptor classes that appear to be functionally linked to the production of NO. Studies have demonstrated the presence and involvement of endothelial muscarinic cholinergic[58-60], kinin[61-63], CGRP[64], substance P[52,65,66], β-adrenergic[31,67,68], and α_2-adrenergic[69] receptors in the production of endothelial-derived NO. These receptors evoke NO synthesis via a variety of signaling mechanisms, involving both convergent and divergent signal transduction cascades. Several of these signaling cascades/mechanisms will be discussed at length below.

Acetylcholine, which evokes endothelial-dependent vasodilation, paradoxically, produces vasoconstriction when the endothelium is absent.[59,60] The muscarinic cholinergic receptors are subdivided into the M_1, M_2, M_3, M_4, and M_5 subtypes.[70] Various animal studies using large arteries have demonstrated that the predominant subtype found on the endothelium is the M_3 receptor.[58,60,71] Stimulation of these M_3 receptors has been shown to cause endothelial-dependent vasorelaxation mediated by release of EDRF.[60] The M_3 is a guanine nucleotide-dependent regulatory proteins (G-protein) linked receptor that activates phospholipase C - phospoinositide hydrolysis, yielding inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG).[70] IP_3, a second messenger, liberates calcium from intracellular stores, which then activates the Ca^{2+}-calmodulin-dependent eNOS.[70] This cascade is thought to be one of the major pathways by which acetylcholine elicits vasorelaxation.

It should also be noted that muscarinic cholinergic receptors have been found to be functionally coupled, in a way not fully understood and involving G-proteins, to K^+ channels.[70] There is increasing evidence that acetylcholine induced vasorelaxation is dependent on activation of the calcium-dependent K^+ channels (K^+_{Ca}), and the resultant increase in Ca^{2+} influx from extracellular sources.[62,72] It is thought that the primary event in acetylcholine-induced vasorelaxation is receptor-mediated IP_3 synthesis leading to an increase in $[Ca^{2+}]_i$. The rise in $[Ca^{2+}]_i$ activates the K^+_{Ca}-channel, producing a "secondary" endothelial cell hyperpolarization.[72] This hyperpolarization of the endothelial cells can then potentiate the rise in $[Ca^{2+}]_i$ by increasing Ca^{2+} influx as a result

of the now enhanced electrochemical gradient. It is likely that all these mechanisms play a role in increasing $[Ca^{2+}]_i$ and thus stimulate NO synthesis (refer to Figure 1).

Kinin receptors, specifically bradykinin, are also linked to endothelial-NO production.[63,73] There are two types of kinin receptors, B_1 and B_2. The endogenous peptide, bradykinin, is a specific agonist for the B_2-receptor, which is G-protein linked and activates the phospholipase C cascade (phospoinositide hydrolysis → producing IP_3 and DAG).[63,73,74] It has been shown in bovine coronary arteries that the predominant kinin receptor subtype constitutively expressed on the endothelium is B_2.[63] Therefore, like the muscarinic cholinergic M_3-receptor, B_2 –receptor stimulation increases $[Ca^{2+}]_i$ via the IP_3 second messenger (see Figure 1). Interestingly, a very recent study by Ju *et al* presented evidence that the endothelial B_2-receptor physically associates with eNOS in a ligand and Ca^{2+}-dependent manner, causing inhibition of the enzyme.[75] In the absence of an endothelial layer bradykinin acts directly on the vascular smooth muscle as a vasoconstrictor[61], possibly by activating both smooth muscle B_1 and B_2-receptors.[76]

In addition to these mechanisms for regulating endothelial-NO production, it is likely that bradykinin-induced stimulation of the endothelium causes smooth muscle hyperpolarization.[77] This hyperpolarization is a result of a cascade identical to that of acetylcholine-induced endothelial hyperpolarization (see above).[77] Both acetylcholine and bradykinin activate phospholipase C and result in similar second messenger cascades. This hyperpolarization, as stated previously, adds to the activation of eNOS.

Endothelial NO production has been functionally linked to β_2–adrenoceptors in several canine studies.[31-34] The β_2-adrenoceptors are a G-protein linked receptor class; G_s activates a membrane bound adenylate cyclase. Activated adenylate cyclase then catalyzes the conversion of ATP to cyclic adenosine monophosphate (cAMP).[70] In addition, a recent study by Ming *et al* found evidence of involvement of ATP-sensitive K^+ channels (K_{ATP}) in β_2-adrenergic coronary vasodilation in dogs.[32] They found that β_2-adrenergic coronary vasodilation was not only NO-dependent, but was also dependent on functioning K_{ATP} channels. This data is supported by several studies demonstrating the existence of K_{ATP} channels on the endothelium.[78,79] Therefore, it appears that the endothelial β_2-adrenoceptor is functionally coupled to K_{ATP} channels and thus stimulation increases K^+ efflux. Potassium ion efflux results in hyperpolarization of the cell and adds to the electrochemical gradient for Ca^{2+} entry. This mechanism could provide for the needed rise in $[Ca^{2+}]_i$ to stimulate eNOS production of NO.

Prostaglandins and Endothelins

In addition to NO, the endothelium synthesizes and releases several other vasoactive substances, the most prominent are prostacyclin (PGI_2) and endothelin. PGI_2 is a derivative of arachidonic acid metabolism, a result of a series of reactions involving, first cyclo-oxygenase, followed by prostacyclin-synthase.[42] PGI_2 release is stimulated by many of the same factors as NO release, these being, shear stress, acetylcholine, bradykinin, catecholamines, and endothelial cell hyperpolarization.[42,80,81]. The

principal vascular activities of PGI_2 include relaxation of vascular smooth muscle and inhibition of platelet adhesion/aggregation. However, under physiologic conditions their contribution to regulation of coronary blood flow is minor. This is evident in the fact that coronary vasomotor tone is not substantially changed by blockade of PGI_2 production.[42] The endothelium also releases the peptide endothelin (ET), of which there are three types: ET_1, ET_2, and ET_3. ET_1 is exclusively produced in the endothelial cell lining, while the others are produced in a variety of tissues.[42] ET_1 is an extremely potent and long lasting vasoconstrictor, that acts directly on the vascular smooth muscle via the ET_A-receptor. Endothelin is synthesized in the endothelium by proteolytic cleavage of "big endothelin" by the ET-converting enzyme. Production and release of ET_1 can be stimulated by shear stress, hypoxia, ischemia, angiotensin II, thrombin, and insulin.[42] Similar to PGI_2, ET_1 appears to play no significant role in regulating coronary blood flow, under normal physiologic conditions.[42]

In summary, the vascular endothelium plays a vital role in modulating coronary vascular tone. Bradykinin, acetylcholine, and norepinephrine, cause constriction of vascular smooth muscle in the absence of the endothelium; however, with an intact endothelial layer, these agents cause vascular smooth muscle relaxation. The role of the endothelium is diverse and it has the potential to interact with most previously discussed control mechanisms of coronary blood flow. These facts make obvious the possible implications of endothelial dysfunction in disease.

Renovascular Hypertension

Renovascular hypertension is a sustained elevation in systemic arterial pressure caused by arterial constriction, stenosis, or lesions in the renal arterial vasculature of one (unilateral) or both (bilateral) kidneys.[1] In renovascular hypertension, increased activity of the renin-angiotensin system exerts long lasting intrarenal and systemic actions. Unilateral renal stenosis is particularly interesting because there appears to be significant interactions between the stenotic and normal kidneys that influence the normal kidney and permit the sustained hypertension[1] (see Figure 2). In the normotensive patient, one kidney is sufficient to maintain fluid and electrolyte balance and arterial pressure; however, in unilateral renal stenosis, the normal kidney does not prevent the development of hypertension.[1] Thus, during unilateral renal artery stenosis the development of hypertension is dependent on how the normal kidney responds and adapts to the progressive changes in the hormonal, neural, and hemodynamic influences that result from the arterial stenosis of the contralateral kidney.

The stenotic kidney responds to the reduced perfusion pressure by increasing production and release of renin into the circulation.[1] As depicted in Figure 3, this enzyme leads to an elevation of circulating angiotensin I and angiotensin II. Angiotensin II has several actions.[1] Angiotensin II causes renal vasoconstriction, reducing renal plasma flow and glomerular filtration rate. Angiotensin II also directly enhances proximal tubule sodium reabsorption. Additionally, angiotensin II stimulates aldosterone secretion resulting in increased sodium reabsorption in the collecting ducts. Angiotensin II is a potent vasoconstrictor as well as a promoter of vascular smooth muscle growth.

Angiotensin II-driven cell growth and replication may contribute to chronic hypertension-induced vascular smooth muscle hypertrophy.[82,83] These actions are mediated through angiotensin II receptors (the subtype AT_1 specifically), which activate the G-protein, phospholipase C, DAG, and IP_3 pathway.[84] Elevated circulating angiotensin II also stimulates the normal kidney (see Figure 2) to increase its local production of angiotensin II.[1] Elevated plasma angiotensin II is known to increase sympathetic neural outflow from the central nervous system (CNS), a property that has been implicated in the maintenance of renovascular hypertension.[85]

The normal kidney, although not an initial causative factor, contributes significantly to the maintenance of hypertension in unilateral renal artery stenosis. The normal kidney is affected in the same manner as the stenosed kidney by the elevated angiotensin II and aldosterone levels. These agents cause the normal kidney to augment sodium reabsorption and thus, blunt the normal pressure natriuretic response to elevated arterial pressure. Systemically, these mechanisms result in increased systemic vascular resistance and augmentation of salt and water reabsorption by the kidneys in an attempt to restore perfusion of the stenosed kidney by increasing arterial blood pressure. However, because the pressure distal to the stenosis is never restored, there is a continual stimulus for renin release from the stenotic kidney.

Renovascular Hypertension - Effects on the Vasculature

Renovascular hypertension, a renin-angiotensin-dependent form of hypertension, is a circulatory disorder characterized by enhanced peripheral vascular resistance which may be the result of structural and functional changes in the blood vessel wall. Prolonged hypertension is known to induce morphological changes in both the endothelium and the vascular smooth muscle cell, including alterations in cell shape, replication rate, and permeability.[43,86] These structural changes lead to vascular hyperplasia and hypertrophy which would tend to promote the effects of vasoconstrictors and attenuate the effects of vasodilators.[43] Thus, vascular adaptations may involve changes in smooth muscle reactivity, vessel compliance, vascular hypertrophy, and/or smooth muscle cell membrane function. However, the augmented vascular reactivity found in hypertension is known to occur during very early and developmental stages, presumably before significant vascular remodeling occurs.[87-89] Therefore, it is likely that structural remodeling of the vasculature is not the only mechanism involved in enhanced peripheral vascular resistance and altered vascular reactivity.

Functional control systems that determine vascular resistance were described in the previous section (i.e., neural, humoral, metabolic, endothelial, etc.). Each of these control mechanisms exerts vasoconstrictor and/or vasodilator influences on the vascular smooth muscle, thereby altering vasomotor tone. In renovascular hypertension, it appears that these mechanisms are altered in some manner, with evidence implicating dysfunctional roles of the sympathetic nervous system and vascular endothelium. Alterations in these control mechanisms lead to exaggerated vasoconstrictor influences

and/or attenuated vasodilatory influences. Potentially, there are numerous mechanisms mediating coronary vascular adaptations to renovascular hypertension. These are depicted in Figure 4.

Sympathetic nervous activity and adrenergic receptor sensitivity appear to be altered in hypertension. Increased vascular reactivity to vasoconstrictors has been found in both clinical[90,91] and experimental[87-89,92,93] forms of hypertension. Several studies have demonstrated that the pressor responses to systemic norepinephrine (combined α_1 and α_2 agonist) and phenylephrine (selective α_1 agonist) are augmented in several different hypertensive animal models.[88,89,94] Additionally, aorta from rats with renovascular hypertension exhibited enhanced constriction to norepinephrine which was augmented by removal of the endothelium.[87] Altered coronary responses have also been identified. Fuchs *et al* reported that α-receptor mediated constriction was exaggerated in isolated coronary arteries from spontaneously hypertensive rats (SHR).[95] These data suggest a general enhancement of α_1-adrenergic constriction which might be revealed by an impaired endothelial-dependent vasodilation. Moreover, it has been found that in hypertension there is an augmentation of the sympathetic stimulation of cardiac function and increased systemic vascular resistance.[96-98] We have recently demonstrated that coronary vascular resistance and vascular reactivity to vasoconstrictor agents are enhanced in the renovascular hypertensive dog; however, the mechanisms for adaptation are not known.[97]

Increased activation of the sympathetic nervous system and the renin-angiotensin system in renovascular hypertension may contribute to the reduced vasodilatory capacity of the coronary vasculature observed. This may occur by increasing vascular responsiveness to norepinephrine (NE), endothelin, and/or angiotensin II, and/or by impairing compensatory vasodilatory influences (β_2-adrenergic and endothelial NO-mediated vasodilation). These changes would augment coronary vasoconstriction and/or decrease the vasodilatory reserve. Such impairments in coronary blood flow control would help explain why hypertensive patients without coronary artery disease or left ventricular hypertrophy often experience angina.[99] During renovascular hypertension, angiotensin II levels are increased. Studies also suggest that elevated sympatho-adrenal activity plays an important role in the pathogenesis of hypertension.[96] Angiotensin II has been shown to a) increase sympathetic outflow from the central nervous system,[100-104] b) increase prejunctional release of norepinephrine (NE),[104] c) increase postjunctional responsivity to NE,[89,94,105-107] d) inhibit neuronal uptake of NE,[104] e) enhance vascular smooth muscle growth[82,83] and f) increase vascular superoxide production.[108,109]

Maintenance of hypertension has been proposed to be due to increased postjunctional sensitivity to adrenergic receptor stimulation in heart and vascular smooth muscle.[86,88,89,105-107,110] However, no conclusive experimental evidence supports this hypothesis, and functional studies in patients and animals have yielded contradictory results.[96,111-114] Our data suggest that the degree of sympathetic activation and

coronary responsivity to adrenergic stimulation are greater in dogs after renovascular hypertension,[97] but the mechanisms mediating this response are unknown.

Another critical factor that regulates vascular tone is the endothelium. Endothelial dysfunction has been implicated in the decreased vasodilatory capacity of the coronary vasculature in hypertension in both clinical[115-119] and experimental[120,121] studies. However, this theory is, at present, controversial. In hypertensive patients, evidence of impaired endothelial-dependent relaxation has been observed in the forearm vascular bed[52] and in the coronary arteries.[115,116,119] Likewise, in hypertensive rats, decreased endothelial-dependent vasodilation has been reported in both large conduit and coronary resistance vessels.[120,122,123] Basal NO production has also been reported to be decreased in patients with essential hypertension,[117] in cultured endothelial cells from hypertensive rats,[124] and in isolated SHR hearts.[125] In support of these observations a recent study reported that eNOS expression was lowered in SHR intramyocardial arterioles with intact endothelium.[120] Conversely, studies in SHR hearts have reported increases in stimulated NO production[121]. Other studies have indicated that in SHR coronary arterioles there was no impairment[95] or even a slight increase[121] in endothelial-dependent vasodilation.

The substances produced by vascular endothelial cells (NO, PGI_2, endothelin, etc.), in response to the previously discussed stimuli, act on the vascular smooth muscle cells in a paracrine fashion. Alteration of these vascular paracrine systems may occur in renovascular hypertension and contribute to increased coronary vascular resistance by

attenuation of vasodilatory influences and/or exaggeration of vasoconstrictor influences. As stated, there is recent clinical and experimental evidence that hypertension results in decreased endothelial NO-mediated dilation, however the mechanisms responsible for this dysfunction are not known. It is important to note that most of these clinical and experimental studies have studied patients with essential hypertension and the SHR (a model of essential hypertension). It is quite possible that differences arise from the different models of hypertension used. Renovascular hypertension is an angiotensin-dependent form of hypertension, meaning that angiotensin II levels may be higher in these patient and animal models. There is recent evidence indicating that angiotensin II stimulates a NADH/NADPH- dependent, membrane-bound oxidase in the vasculature and that this is a primary source of superoxide free radicals.[108,109] Laursen *et al* found that angiotensin II-induced, but not NE-induced, hypertension in rats caused increased vascular superoxide formation.[108] In the angiotensin II-induced, but not in the NE-induced hypertension, they also reported impaired dilatory responses to both nitroprusside and acetylcholine in *in vitro* and *in vivo* studies.[108,109] It was concluded by these researchers that the increased superoxide produced locally in the vasculature was responsible for increased degradation of endothelium-derived NO, thus the decreased responses to both acetylcholine and nitroprusside (an exogenous NO-donor). This increased superoxide production and its accompanied endothelial dysfunction were observed after only five days of hypertension. Therefore, it is possible that in forms of hypertension with elevated angiotensin II this mechanism may contribute to vascular disease.

NO produced from the endothelium may have a buffering effect on α-adrenergic constriction;[87] however, as indicated above it is possible that in the hypertensive patient this "protective" mechanism is attenuated or absent. We propose that renovascular hypertension results in damage to the coronary vascular endothelium-dependent vasodilatory mechanisms such that NO buffering of a coronary constrictor tone is reduced. In order to minimize the confounding aspects that left ventricular hypertrophy can have on deciphering the changes that occur in hypertension, we have examined the effects of short term (2-3 weeks) hypertension on the endothelial control of coronary blood flow. In addition, it is not fully known if changes in endothelial function occur in asymptomatic patients with mild hypertension. A very recent study found that indeed, young healthy asymptomatic men with borderline hypertension (systolic 140-160 mmHg or diastolic 90-100 mmHg, or both) exhibited reduced coronary vasoreactivity.[126] However, research needs to be done to examine whether this dysfunction is a result of a general endothelial abnormality or rather specific to various receptors/second messengers. We have examined these possibilities in the coronary vasculature of conscious dogs with short term renovascular hypertension.

The following studies were performed to 1) evaluate the integrity of endothelial-independent and –dependent coronary vasodilation and 2) evaluate the integrity of β-adrenergic mediated coronary vasodilation. These studies were performed in the same dogs before and after inducing renovascular hypertension, therefore each dog serves as its own control.

In the following studies we have tested two main hypotheses:

1) The reduced coronary vasodilatory capacity found in renovascular hypertension is due, in part, to an impaired endothelial-dependent vasodilation.

2) The reduced coronary vasodilatory capacity found in renovascular hypertension is due, in part, to an impaired β-adrenergic mediated vasodilation.

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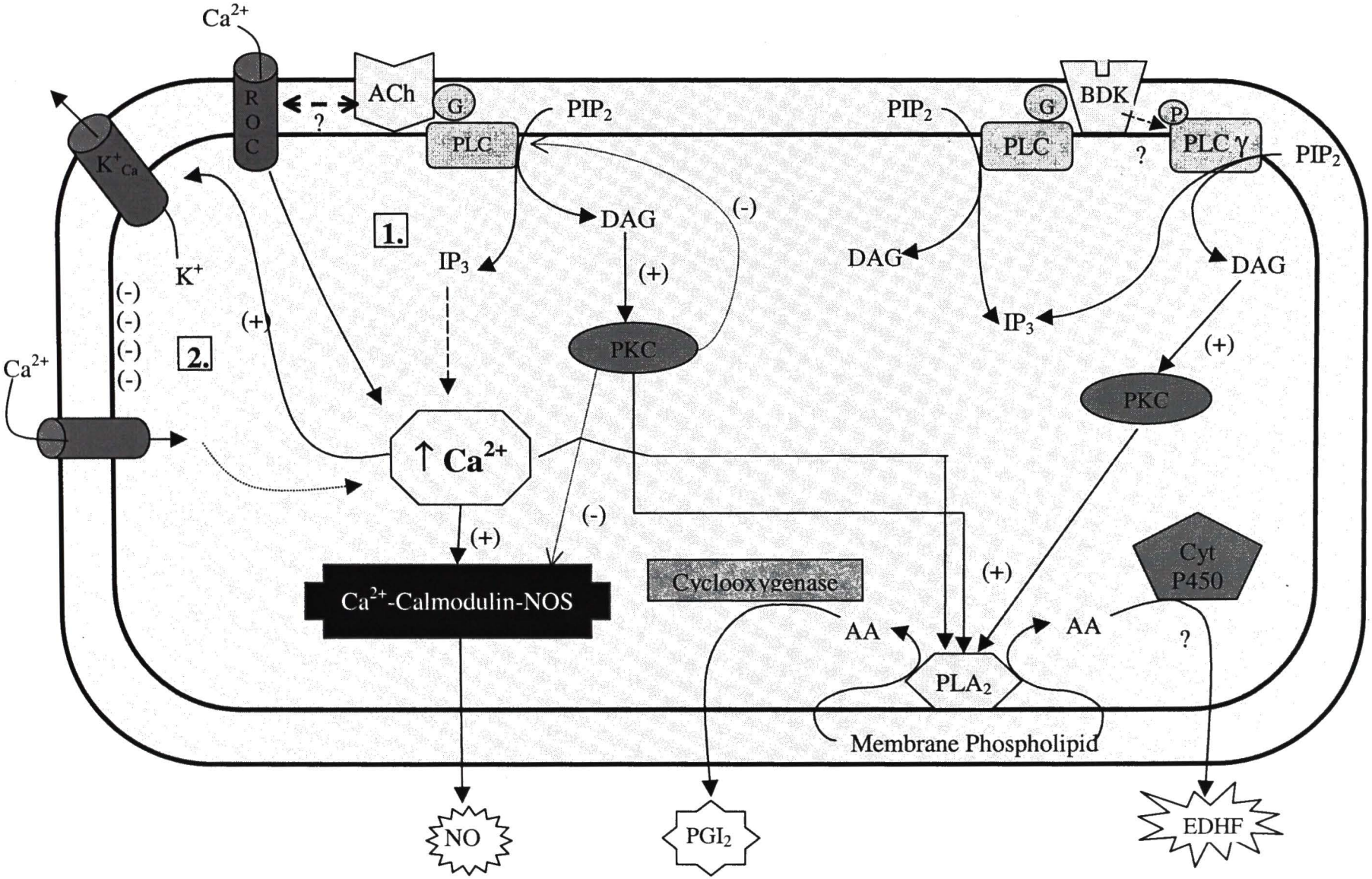


Figure 1. Signal transduction pathways for acetylcholine (ACh) and bradykinin (BDK) in a coronary endothelial cell leading to production of vasodilator substances. Abbreviations: PIP_2, phosphoinositide bisphosphate; DAG, diacylglycerol; IP_3, inositol triphosphate; PLC, phospholipase C; PKC, protein kinase C; ROC, receptor-operated Ca^{2+} channel; K^+_{Ca}, Ca^{2+} sensitive K^+ channel; PLA_2, phospholipase A_2; Cyt P450, cytochrome P450 oxidase; AA, arachidonic acid; PGI_2, prostacyclin; EDHF, endothelial-derived hyperpolarizing factor; G, G-protein. (1) Primary rise in intracellular Ca^{2+} due to IP_3 and ROC activation. (2) secondary rise in intracelluar Ca^{2+} due to activation of K^+_{Ca} and subsequent membrane hyperpolarization leading to increased Ca^{2+} influx.

Figure 2. Local and systemic effects of unilateral renal artery stenosis

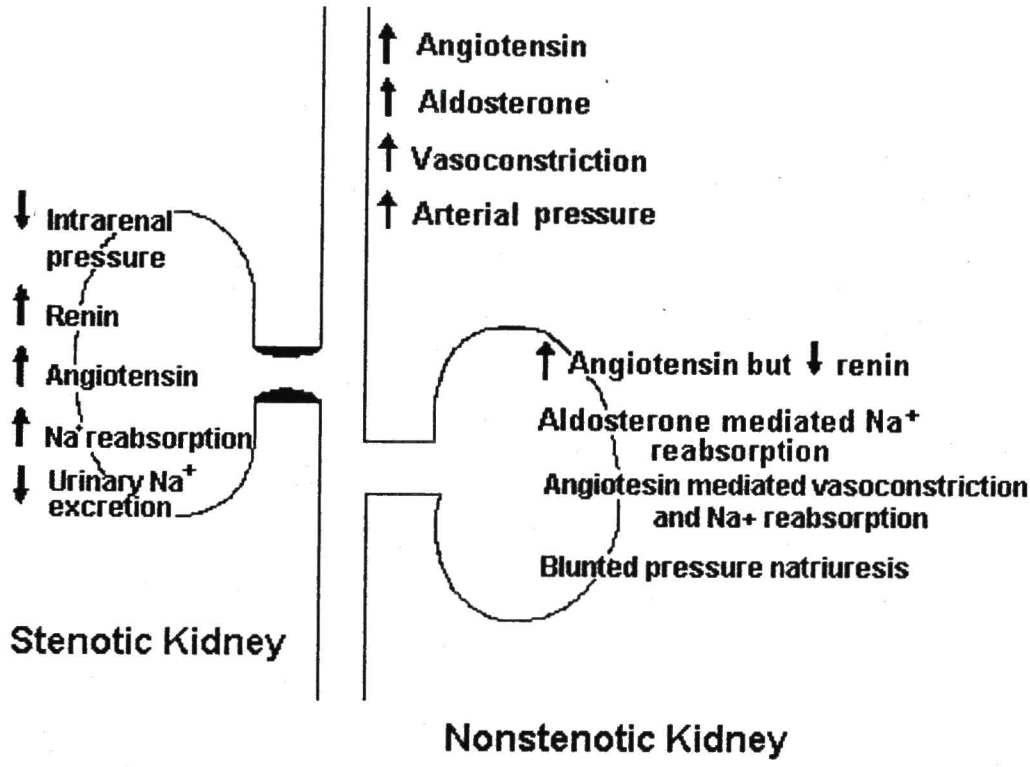


Figure 3. The Renin-Angiotensin-Aldosterone System.

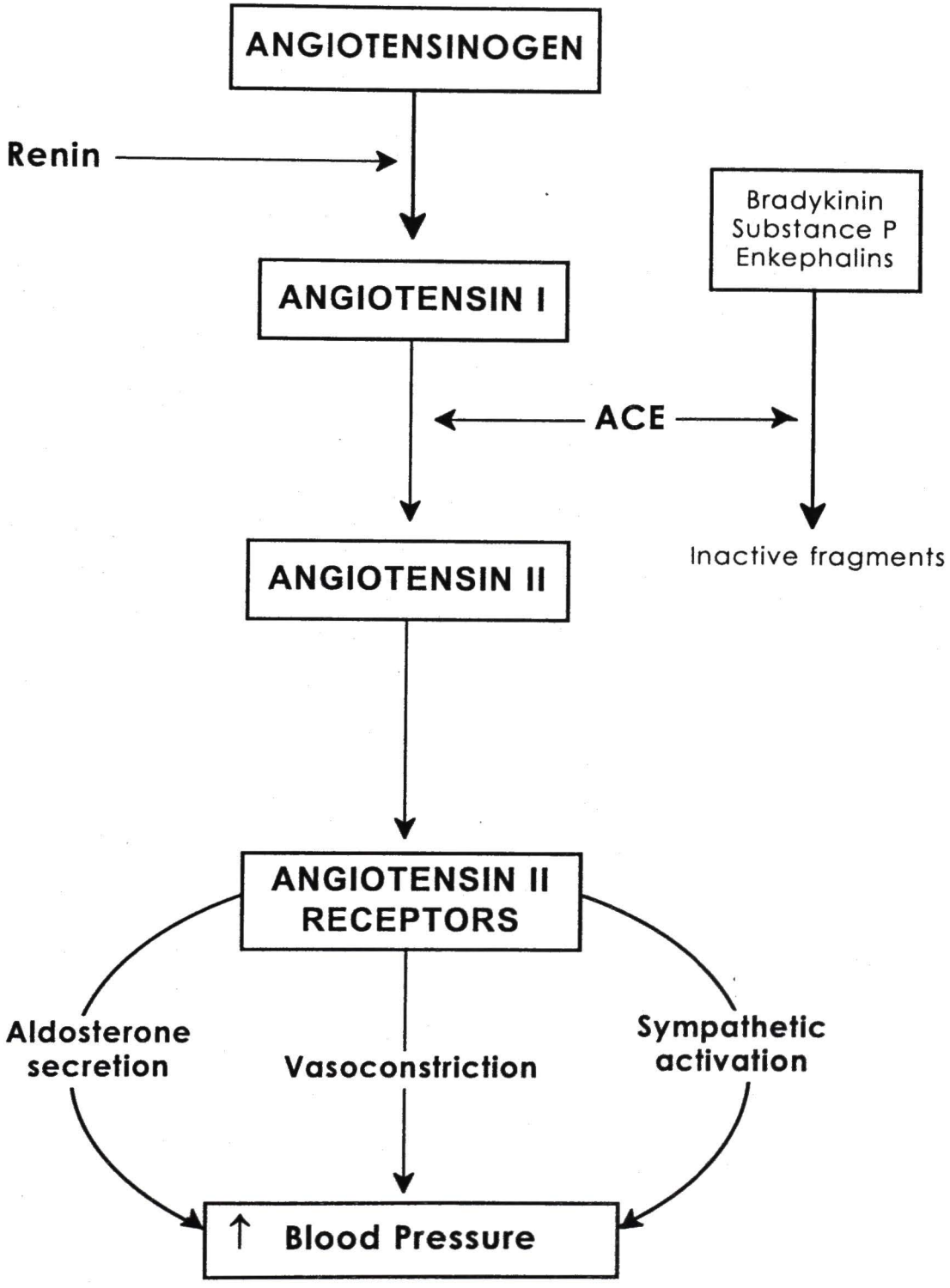
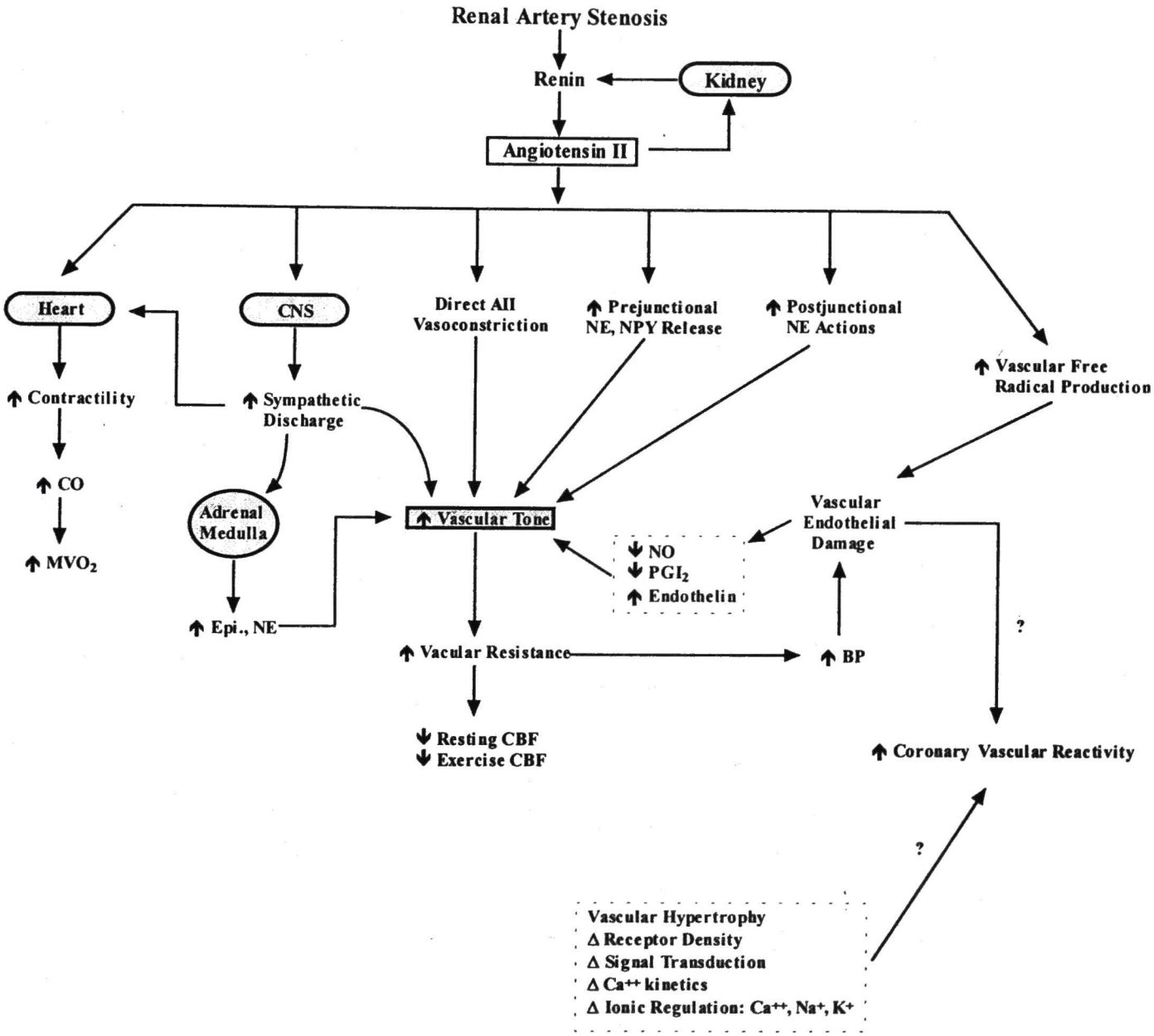


Figure 4. Potential Mechanisms Affecting Vascular Tone in Renovascular Hypertension



CHAPTER II

EARLY IMPAIRMENT OF ENDOTHELIAL-DEPENDENT CORONARY VASODILATION IN CONSCIOUS DOGS WITH RENOVASCULAR HYPERTENSION

Geoffrey P. Kline and Patricia A. Gwirtz

Department of Integrative Physiology

University of North Texas Health Science Center

Fort Worth, Texas 76107-2699

ABSTRACT

The present studies were conducted to test the hypothesis that the reduced coronary vasodilatory capacity in renovascular hypertension (RVH) is mediated by impaired endothelial function. We evaluated coronary vascular responses to intracoronary (i.c.) bolus injections of graded doses of the endothelial-dependent vasodilators acetylcholine (ACh, 0.1 to 1.0 μg) and bradykinin (BDK, 0.005 to 0.1 μg) and the smooth muscle dilator sodium nitroprusside (SNP, 10 to 80 μg) in chronically instrumented dogs. ACh (0.5 μg) increased CBF by 107±8 ml/min, BDK (0.02) increased CBF by 80±14 ml/min, and SNP (40 μg) increased CBF by 120±14 ml/min. After the administration of i.c. Nω-nitro-L-arginine (LNA, 55-75 mg over 25 min) to block nitric oxide (NO) synthesis, baseline CBF was not altered, and CBF increases to ACh and BDK were significantly reduced ($P<0.05$). After RVH, resting CBF was reduced from 63±5 ml/min to 53±6 ml/min ($P<0.05$). After RVH, ACh (0.5 μg) increased CBF by 74±11 ml/min, BDK (0.02 μg) increased CBF by 46±8 ml/min, and SNP (40 μg) increased CBF 88±13 ml/min, all significantly less than before RVH ($P<0.05$). LNA administration after RVH further reduced resting CBF to 43±6 ml/min ($P<0.05$). Interestingly, LNA after RVH further attenuated increases in CBF to i.c. administration of BDK ($P<0.05$), but not to ACh. Therefore, after RVH both the endothelial-dependent (ACh and BDK) and endothelial-independent (SNP) coronary vasodilations were impaired. These results indicate that coronary dysfunction during RVH is not isolated to the endothelium.

Furthermore, after RVH in this study, NO was not involved in ACh-induced dilation, whereas it was involved in BDK-induced dilation.

Keywords: Coronary circulation, endothelium, renovascular hypertension, nitric oxide

INTRODUCTION

Hypertension is frequently associated with increased coronary vascular resistance and reduced vasodilatory capacity, even in the absence of left ventricular hypertrophy. [1-4] The mechanisms responsible for impaired coronary vasodilatory capacity in systemic hypertension are unclear.

Endothelial-dependent responses to various vasoactive substances have been demonstrated in several vascular beds. Studies in patients[2,5,6] and animal models[7,8] of hypertension suggest that a dysfunctional endothelium, resulting in abnormal endothelial-dependent vasodilation, contributes to the impaired coronary dilatory capacity. Endothelium-dependent relaxation appears to be depressed in the aorta and large arteries of various models of hypertension,[2,7,9-11] while endothelial-independent vasodilation is preserved.[2,5,12,13]

While studies have demonstrated an important role of the endothelium in responses of large coronary arteries, few studies have examined whether impairment of the coronary vascular endothelium is responsible for increased coronary resistance and decreased dilatory capacity, especially in an intact coronary circulation. Reports in the

literature conflict regarding whether or not systemic hypertension damages the coronary vascular endothelium. Tschudi *et al* found normal endothelial function in the coronary vasculature of spontaneous hypertensive rats (SHR).[14] Other labs using coronary vessels from SHR have reported similar findings.[12,15,16] Preserved endothelial-dependent vasodilation has also been observed in patients with essential hypertension.[17] In contrast, others have found impaired endothelial-mediated regulation of coronary vascular tone in patients with essential hypertension[2,5,6] and various animal models of hypertension.[7,8]

The present studies were conducted to test the hypothesis that the reduced coronary vasodilatory capacity in renovascular hypertension (RVH) is mediated by impaired endothelial function after hypertension. We tested this hypothesis by evaluating coronary vascular responses to intracoronary administration of the endothelial-dependent vasodilators acetylcholine and bradykinin and the smooth muscle dilator sodium nitroprusside in chronically instrumented dogs before and after RVH. We also examined the role of nitric oxide (NO) in the vasodilatory responses to these agents, before and after RVH, by inhibiting NO synthesis with the L-arginine analog, Nω-nitro-L-arginine (LNA).

MATERIAL AND METHODS

Surgical Preparation

Experiments were performed on six (6) healthy, mongrel dogs of either sex (weight range 25-35 kg). All dogs were premedicated with acepromazine (0.03 mg/kg,

s.c.) and anesthetized with thiopental sodium (5 mg/kg, i.v.). The trachea was intubated and anesthesia maintained with isoflurane gas (1-3%) with an equal offset of O_2 (1 L). Using sterile technique, a thoracotomy was performed through the left fifth intercostal space and the heart instrumented as previously described in detail.[18]

The aorta was exposed and a fluid-filled Tygon catheter was inserted just distal to the aortic arch to monitor aortic pressure (AoP). A Transonic transit time Doppler flow probe (Transonic Systems Inc, Ithaca, NY) was placed around the root of the aorta to measure cardiac output (CO). For the measurement of left ventricular pressure (LVP) a Konigsberg P-6.5 transducer solid state micromanometer (Konigsberg Instruments Inc, Pasadena, CA) and a fluid-filled Tygon catheter were inserted into the left ventricle through a stab-wound in the apex. The Konigsberg pressure transducer was calibrated before implantation and routinely checked and adjusted by simultaneously measuring pressure with the implanted Tygon catheter connected to an external Isotec® pressure transducer, which was calibrated using a mercury manometer. The circumflex artery was dissected free of the surrounding tissue for a distance of approximately 3 cm beginning at the origin of the vessel. A 10 MHz Doppler ultrasonic flow probe (4 mm ID) was positioned around the circumflex artery for measurement of circumflex blood flow velocity (CFV) using a Triton Flowmeter (San Diego, CA). At necropsy, the internal circumference of the vessel under the probe was measured to obtain the vessel cross-sectional area which was used to convert CFV to coronary blood flow (CBF). To check the zero flow reference, a pneumatic occluder was placed around the circumflex artery immediately distal to the Doppler ultrasonic flow probe such that there was no vessel

branch between the two. The circumflex artery was cannulated with a heparin-filled Silastic catheter distal to the occluder for injection of the solutions into the circumflex artery.[19]

After instrumentation was completed, a chest tube was placed in the thoracic cavity to evacuate the pneumothorax and any post-surgical intrathoracic exudate accumulation. All wires and catheters were tunneled subcutaneously to exit between the scapula. Indwelling catheters were flushed daily with heparinized saline to maintain patency. Post-operative analgesics, antibiotics, and antipyretics were given by the veterinarian for 5 days, or as needed. At least 10 to 14 days were allowed to elapse before experiments were begun. During this time the dogs were familiarized to the laboratory setting.

After initial control (normotensive) studies were performed, the dogs were re-anesthetized as described above and a midline laparotomy performed. Both the left and right renal arteries were isolated and 10-MHz Doppler flow probes and saline filled occluders were placed around the vessels. The incision was then closed in layers and occluder and lead wires were tunneled subcutaneously to exit between the scapula. All protocols were reviewed and approved by the institutional Animal Care and Use Committee.

Model of Renovascular Hypertension

Hypertension was induced by the unilateral renal stenosis method (two-kidney, one clip Goldblatt hypertension model) described by Anderson *et al.*[20] This model is

more appropriate for the study of renovascular hypertension (RVH) than the one-kidney models, which are more appropriate for the study of renal parenchymal hypertension.[21] After prestenosis, or normotensive, studies were completed one of the renal arteries was stenosed by inflating the saline filled occluder until renal flow was reduced by 60%.[22] Verification of stenosis was performed several times daily. Studies were repeated 2 weeks after a stable hypertensive state was achieved. Blood pressure was monitored on a daily basis during developing and stable states of hypertension.

Experimental Protocol

In all experiments, the dogs were trained to lay quietly on the table in a calm, resting manner. Studies were performed only if resting HR was ≤ 90 bpm, to ensure a resting state.

Normotensive Studies. Experiments were performed 2 weeks after initial surgery to examine the coronary vascular reactivity to endothelial-dependent and independent vasodilators and to determine the relative importance of NO in these responses. After resting control measurements, dose-coronary flow responses to several vasodilator agents were obtained. Bolus intracoronary (i.c.) injections of the endothelial-dependent vasodilators acetylcholine (ACh) (0.1, 0.2, 0.5, 0.75, 1.0 μg) and bradykinin (BDK) (0.005, 0.01, 0.02, 0.05, 0.1 μg) were made. Bolus i.c. injections of the endothelial-independent vasodilator sodium nitroprusside (SNP) (10, 20, 40, 80 μg) were also made. During these experiments all hemodynamic variables were continuously recorded and monitored. Drugs and doses were given in a randomized order. Doses were selected to

produce dose-dependent increases in CBF without affecting other hemodynamic variables. All drugs were dissolved in sterile saline and then filtered with sterile Acrodisc (0.2 μm) filters (Gelman Sciences) on the day of the experiment. Volume of the drugs injected were ≤1.0 ml and were followed by a saline flush of 2 ml over 18 seconds. In each dog it was verified that a vehicle flush of this volume and speed did not alter CBF. CBF data reported was measured at the peak response to each drug. CBF was allowed to return to baseline for several minutes before administering the next dose. On a separate day, dose responses to ACh, BDK, and SNP were repeated after endothelial NO synthesis was inhibited by an i.c. infusion of the L-arginine analog Nω-nitro-L-arginine (LNA) (55-75 mg over 25 min).

Hypertensive Studies. After normotensive studies were completed, all dogs were made hypertensive using the method described above; hence each dog served as its own control. Two weeks into the hypertensive state, the protocols described above were repeated. Once again dose responses to ACh, BDK, and SNP were performed in the same manner as those in the normotensive state. These responses were also repeated after NO synthesis blockade with LNA.

Data Collection and Analysis

Signals were simultaneously recorded on a Coulbourn 8-channel paper chart recorder (Allentown, PA) and a 15-channel Vetter Model 4000A PCM recording adaptor (A.R. Vetter, Rebersburg, PA) for digital recording with a Vetter-modified Model 500 VCR . Signals were also sent to an IBM compatible computer using a PowerLab/800

(ADInstruments) system. All data were recorded in real-time and later analyzed using the computer analysis program Chart (v3.4) for Windows (ADInstruments). The following data were analyzed from the recorded variables: left ventricular systolic pressure (LVSP), maximum rate of left ventricular pressure development ($+dP/dt_{max}$), heart rate (HR), mean aortic pressure (AOP), CO, and CFV.

Data are reported as mean ± standard error of the mean (SEM), and differences between means were considered statistically significant if the probability of their occurring by chance was less than 5% ($p < 0.05$). Multiple simultaneous comparisons of baseline data in control conditions, after LNA, after RVH, and after RVH+LNA were made using an analysis of variance for repeated measures followed by Student-Newman-Keuls test to isolate specific contrasts. Comparisons of drug responses before and after LNA and before and after RVH were made using paired *t* tests.

RESULTS

Baseline Hemodynamics

RVH resulted in a significant increase in mean AOP and LVSP and significant decreases in CBF, HR and CO (see Table 1). There was no change in resting $+dP/dt_{max}$, although there was a trend for it to be increased. In order to examine the role of coronary endothelial NO in mediating the vasodilatory responses to the vasoactive agents used in this study, endothelial nitric oxide synthesis was inhibited using LNA. The hemodynamic effects of i.c. LNA are also shown in Table 1. NO blockade resulted in a

small but significant increase in mean AOP and LVSP in the normotensive state, suggesting that a small spillover of LNA into the systemic circulation occurred. There was no change in $+dP/dt_{max}$, CO, HR or CBF after NO blockade with LNA in normotensive state. In contrast, LNA resulted in a significant decrease in CBF in the hypertensive state, with no changes in mean AOP, LVSP, $+dP/dt_{max}$, CO or HR. With the exception of CBF, i.c. bolus injections of ACh, BDK and SNP had no significant hemodynamic effects, indicating no significant systemic spillover of these drugs during the dose-coronary flow response challenges.

Responses to Acetylcholine

As shown in Figures 1-A, 2-A, and 3-A, all dose-CBF response data are reported as change in CBF from the baseline levels presented in Table 1. Likewise, Figures 1-B, 2-B, and 3-B are dose-responses reported as changes in coronary conductance from baseline. Dose-response curves for ACh before and after RVH, in the presence and absence of NO synthase blockade with LNA are shown in Figure 1-A and 1-B. Bolus injections of ACh, i.c., caused dose-dependent increases in CBF and coronary conductance. In the normotensive state, 0.1, 0.2, 0.5, 0.75 and 1.0 μg doses of ACh increased CBF above baseline by 113, 137, 169, 173, and 192 percent, respectively. In the normotensive state, NO blockade significantly attenuated the vasodilatory responses to i.c. ACh at all doses.

Dose-responses were repeated in all dogs after RVH was induced. When compared to the normotensive state, the vasodilatory responses to ACh were significantly

reduced, such that the same doses resulted in 97, 106, 141, 151, 183 percent increases over baseline, respectively. In contrast to the normotensive state, there was no attenuation of the vasodilatory responses to ACh after NO blockade. These data suggest that NO has little or no role in ACh-induced vasodilation in the dog after RVH.

Responses to Bradykinin

Dose-response curves for BDK before and after RVH, in the presence and absence of NO synthase blockade with LNA are shown in Figure 2-A and 2-B. Bolus injections of BDK, i.c., also caused dose-dependent increases in CBF and coronary conductance. In the normotensive state, 0.005, 0.01, 0.02, 0.05 and 0.1 μg doses of BDK significantly increased CBF above baseline by 57, 96, 126, 151, and 167 percent, respectively. In the normotensive state, NO blockade significantly attenuated the vasodilatory responses to i.c. BDK at all doses.

Dose-responses were repeated in all dogs two weeks after induction of RVH . The BDK-induced vasodilation was significantly reduced compared to the normotensive state. Thus, the same doses resulted in 42, 70, 87, 106, and 138 percent increases over baseline, respectively. Similarly to the normotensive state, the vasodilatory response to BDK after NO blockade was significantly reduced at the 0.01 and 0.02 μg doses. These data suggest that unlike ACh, BDK-induced coronary dilation after RVH continues to have a NO-dependent component at the lower doses.

Responses to Sodium Nitroprusside

Dose response curves for SNP before and after RVH, in the presence and absence of NO synthase blockade with LNA are shown in Figure 3-A and 3-B. Bolus injections of SNP, i.c., caused dose-dependent increases in CBF and coronary conductance. In the normotensive state 10, 20, 40, 80 μg of SNP resulted in significant increases in CBF above baseline by 125, 157, 189, and 212 percent, respectively. In the normotensive state, NO blockade significantly augmented the vasodilatory responses to 10, 20, & 40 μg of i.c. SNP.

Dose-responses were repeated in all dogs 2 weeks after induction of RVH. When compared to the normotensive state, SNP-induced vasodilation was significantly reduced. Thus, the same doses resulted in 95, 131, 168, and 175 percent increases over baseline, respectively. In contrast to the normotensive state, NO blockade did not significantly change the vasodilatory response to SNP after RVH.

DISCUSSION

This is the first study to examine the effects of short-term RVH on coronary responsivity to the endothelial-dependent vasodilators ACh and BDK and the endothelial-independent vasodilator SNP in a conscious dog model. The use of the chronically instrumented dog model allows assessment of coronary vascular reactivity in the same dog before and after induction of RVH, thus increasing the power of the study. This approach allows simultaneous evaluation of coronary blood flow and left ventricular

inotropic function. In the present study we investigated the contribution of the coronary vascular endothelium to the impairment of coronary vasodilatory capacity in RVH.

The major results of this study were 1) ACh-induced and BDK-induced coronary vasodilation are impaired following 2 weeks of RVH; 2) SNP-induced coronary vasodilation is also impaired after RVH; 3) the data obtained after NO-blockade suggest that after RVH, ACh-induced coronary vasodilation is relatively independent of NO; in contrast, BDK vasodilation (at least at lower doses) appears to retain an NO-dependent component. Thus, these data indicate that there is an early impairment of coronary vasodilation in RVH to both endothelial-dependent and –independent vasodilators before left ventricular and coronary vascular remodeling (hypertrophy) is apparent. It also appears that the mechanisms responsible for the decreased ACh and BDK responses may not be identical. The attenuated response to the NO donor, SNP, after RVH implicates a mechanism other than one involving the endothelium in the impaired vasodilatory response, as well.

Effects of renal artery stenosis

RVH is a clinically prominent form of secondary hypertension which is associated with elevated levels of circulating angiotensin II. During the early phase of two kidney-one clip RVH the pathogenesis of the disease has been attributed to a combination of elevated angiotensin II, altered plasma volume, changes in renal function, and increased renal nerve activity.[20,23] In the present study, unilateral renal artery

stenosis caused a significant 20 mmHg increase in resting mean AOP and LVSP. Resting CO, HR, and CBF were simultaneously reduced.

Mechanisms of impaired responses during RVH

Several myocardial and vascular factors could be responsible for the early impairment of coronary vasodilation to ACh, BDK, and SNP we observed in dogs with RVH. Because the effects of brief hypertension were examined early after renal artery stenosis (2 weeks), left ventricular hypertrophy and vessel structural changes should be minimal, and therefore have a unlikely role in the observed impaired coronary vasodilation. Potential mechanisms that may have contributed to our observations include increased extravascular compressive forces, augmented constrictor influences, and/or an impairment of dilator influences (particularly those mediated through the endothelium).

Increased vascular reactivity to vasoconstrictors has been found in both clinical[24,25] and experimental[26-30] forms of hypertension. Several studies have demonstrated that the pressor responses to systemic norepinephrine and phenylephrine are augmented in several different models of hypertensive animals.[27,30,31] The aorta from rats with RVH have been shown to exhibit enhanced constriction to norepinephrine and that removal of the endothelium augments this response.[26] Altered coronary responses have also be identified. Fuchs *et al* reported that isolated coronary arteries from spontaneously hypertensive rats (SHR) exhibited exaggerated α-receptor mediated constriction.[12] Recently, our laboratory has reported that coronary vascular resistance and vascular reactivity to vasoconstrictor agents are enhanced in the renovascular

hypertensive dog.[32] These data suggest a general enhancement of α_1-adrenergic constriction in hypertension which might be revealed or exaggerated by impaired endothelial-dependent vasodilation.

The endothelial-dependent regulation of coronary vascular tone may be altered in hypertension by changes in the endothelial vasodilator as well as vasoconstrictor systems. Reduced responses to endothelial-dependent vasodilators could involve a specific defect in all or some receptor-pathways, including an impairment in NO production, a reduction in vascular smooth muscle responsiveness to endogenous NO, increased degradation or inactivation of nitric oxide, and/or a simultaneous formation and release of endothelial-contracting factors that interfere with NO relaxation.

Studies in a variety of tissues and species have demonstrated that the endothelial dysfunction observed after hypertension is not isolated to a specific receptor, rather the endothelial dysfunction is due to a more generalized defect in the endothelial production and release of NO.[2,7,9,10] However, at the same time, and often using the same model, studies have found preserved or even enhanced responses to certain endothelial-dependent vasodilators,[15,16,33] indicating that endothelial impairment in hypertension does not involve every intracellular pathway. Our data support this idea. In the present study, ACh-induced coronary vasodilation was not further reduced in RVH dogs after inhibiting NO-synthesis with LNA, while the vasodilation to BDK was further reduced. These data suggest that after induction of RVH, ACh-induced vasodilation was apparently independent of NO, while BDK-induced vasodilation was dependent, in part, on NO.

Another possible mechanism by which endothelial-dependent vasodilation may be impaired is by a decreased sensitivity of the vascular smooth muscle to NO. A reduced responsiveness to NO donors like SNP has been demonstrated in the aorta and carotid arteries of the hypertensive rat.[34,35] However, other studies have also shown that the vascular response to nitro-vasodilators appears to be preserved in hypertension.[2,5,12,13] Our data indicate that in the RVH dog, the coronary vasodilatory response to SNP is impaired, when compared to normotensive state. Unlike many forms of hypertension, circulating angiotensin II levels are elevated in RVH. Recent studies have indicated that angiotensin II stimulates a NADH/NADPH- dependent, membrane-bound oxidase in the vasculature and that this is a primary source of superoxide free radicals.[36,37] Laursen *et al* found that in angiotensin II-induced, but not norepinephrine-induced, hypertension in rats caused increased vascular superoxide formation.[37] In the angiotensin II-induced, but not in the norepinephrine-induced hypertension, they reported impaired dilatory responses to both SNP and ACh in *in vitro* and *in vivo* studies.[36,37] Thus, increased local superoxide production may be responsible for increased degradation of endothelium-derived NO, thus decreasing responses to both ACh and SNP (an exogenous NO-donor). In addition, since this mechanism was observed after only five days of hypertension, it is therefore possible that in forms of hypertension with elevated angiotensin II this mechanism may contribute to vascular dysfunction early in the disease.

The responses we observed to ACh and BDK were not completely abolished by NO-blockade with i.c. LNA infusion. There are two possible explanations for this

observation, either LNA infusion resulted in an incomplete coronary vascular blockade of NO synthesis or ACh and BDK vasodilation involve other mechanisms in addition to NO formation. Recent studies in dogs found that the ACh-induced coronary vasodilation that persists after NO-blockade is due to the action of a cytochrome P-450 metabolite, most likely endothelial derived hyperpolarizing factor (EDHF).[38,39] ACh and BDK also stimulate the release of the dilator prostacyclin (PGI_2), which is an arachidonic acid metabolite that is also persistent after NO-blockade.[40,41] The impaired ACh vasodilation after hypertension has also been attributed to the increased formation of the endothelium-dependent contracting factor, prostaglandin H_2 in SHR aorta.[33] Our data indicate that ACh vasodilation in RVH is relatively independent of NO, whereas BDK is not, indicating that after RVH the endothelium retains the ability to produce NO to some stimuli. These differences to ACh and BDK could be due to the different receptor pathways involved (muscarinic and kinin, respectively) and/or altered release of either endothelial-derived dilator or constrictor substances.

In summary, our data indicate that after two-weeks of RVH the coronary vasculature exhibits an impaired reactivity to both nitro-vasodilators and the endothelial-dependent vasodilators ACh and BDK. We have also shown that the endothelium retains some ability to produce NO under certain receptor-mediated stimuli after RVH. In the present study, we examined CBF responses that predominantly represent microcirculatory function; therefore, it is likely that the changes observed are due to alterations in resistance vessels. It is important to note that these impaired responses are evident early in the disease process and are present before significant vascular and

myocardial structural changes are thought to occur. Therefore, in the RVH patient it is possible that coronary vasoreactivity is impaired very early in the disease, before any structural changes occur in the cardiovascular system.

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Table 1. Baseline hemodynamic values before and after nitric oxide synthesis blockade in the normotensive and RVH conditions

	Normotensive State		Hypertensive State	
	Baseline	NO-Blockade	Baseline	NO-Blockade
LVSP (mm Hg)	121±5	130±5‡	148±6†	151±5*
$+dP/dt_{max}$ (mm Hg/sec)	2861±223	2749±236	3532±560	3544±592
Mean AOP (mm Hg)	93±2	104±3‡	113±6†	115±6*
CO (L/min)	4.1±0.5	3.8±0.5	3.6±0.4*	3.2±0.5*
HR (bpm)	78±2	72±5	64±4*	61±6*
CBF (ml/min)	63.4±5.0	56.2±4.8	52.5±5.7*	43.0±6.1*‡

Abbreviations: LVSP indicates left ventricular systolic pressure; $+dP/dt_{max}$, maximum rate of left ventricular pressure generation; AOP, aortic pressure; CO, cardiac output; HR, heart rate; and CBF, coronary blood flow; NO, nitric oxide. Values are ±SEM; n=6. NO synthesis blockade was induced by intracoronary infusion of Nω-nitro-L-arginine.

*P<0.05 compared with normotensive. †P<0.01 compared with normotensive.

‡P<0.05 compared with respective baseline.

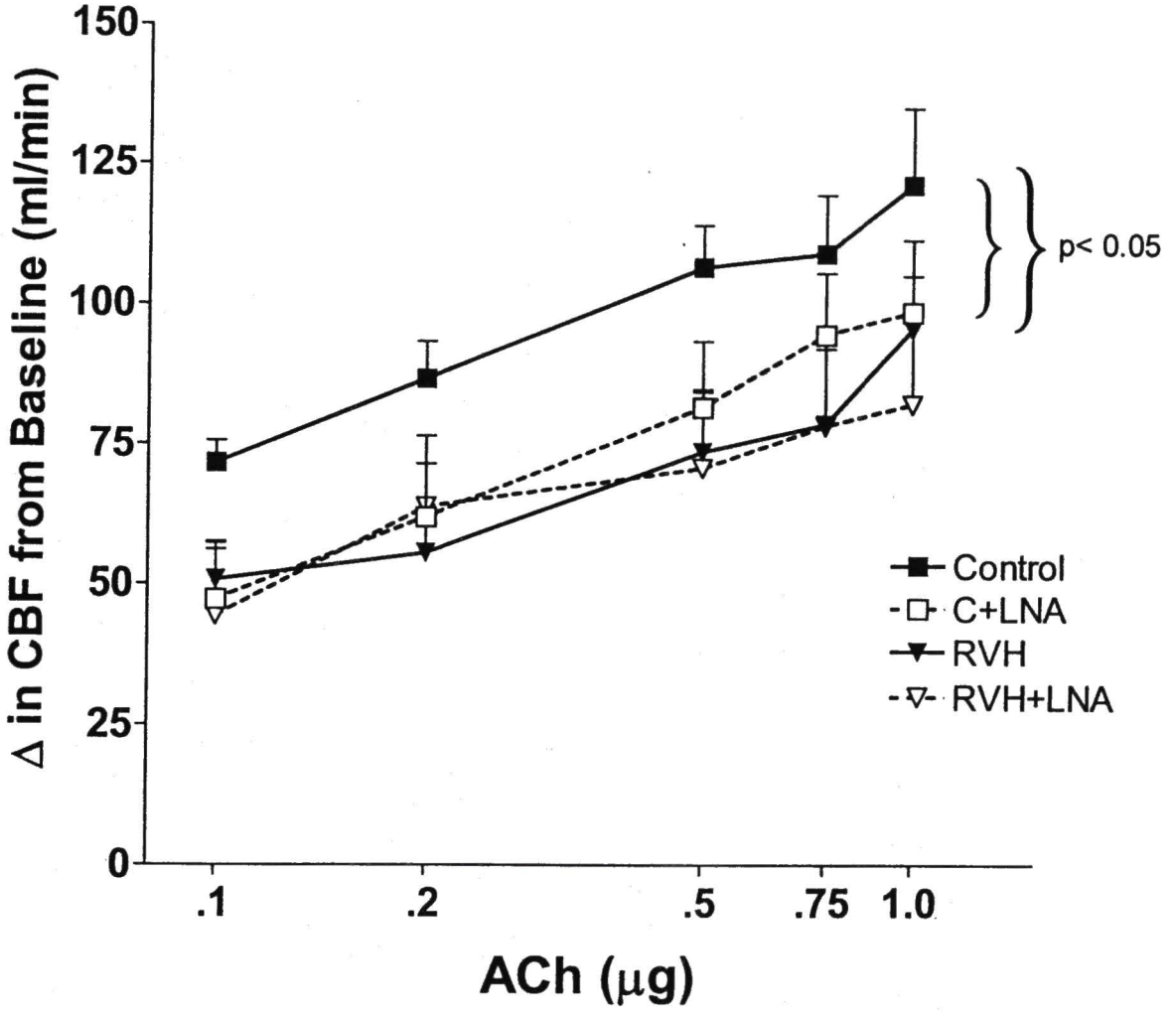

Figure 1-A. Increases in CBF from baseline in response to intracoronary acetylcholine (ACh) before and after NO-blockade with Nω-nitro-L-arginine (LNA) in the normotensive (control) and renovascular hypertensive (RVH) states. In the control condition, LNA significantly reduced ($P<0.05$) the CBF response at all doses (Control vs. C+LNA). RVH resulted in a significantly ($P<0.05$) reduced vasodilation to ACh at all doses, when compared to Control. LNA did not further attenuate the CBF response to ACh in the RVH state. Values are mean±SEM for 6 dogs.

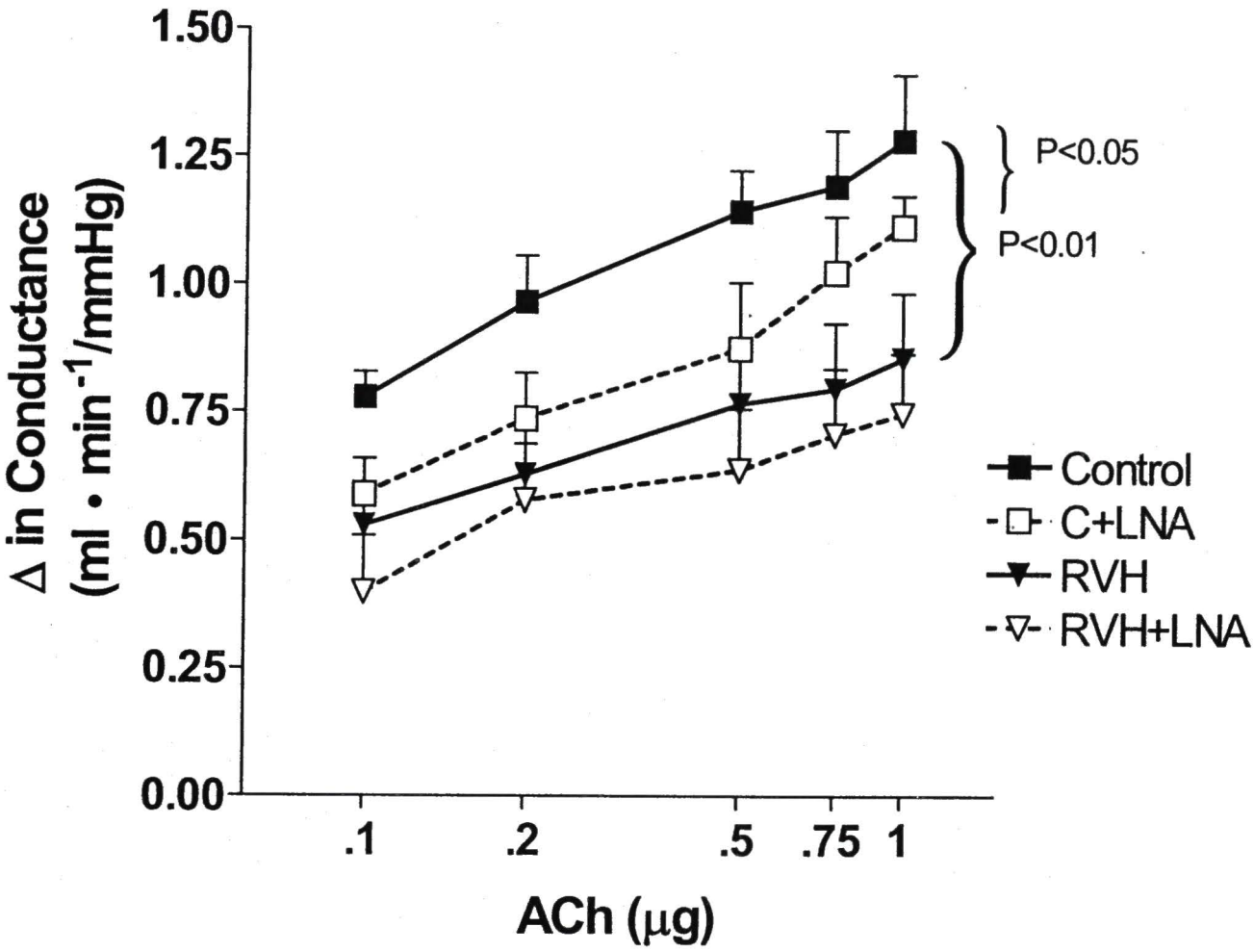

Figure 1-B. Increases in coronary conductance from baseline in response to intracoronary acetylcholine (ACh) before and after NO-blockade with Nω-nitro-L-arginine (LNA) in the normotensive (control) and renovascular hypertensive (RVH) states. In the control condition, LNA significantly reduced ($P<0.05$) the responses at all doses (Control vs. C+LNA). RVH significantly ($P<0.01$) reduced vasodilation to ACh at all doses, when compared to Control. LNA did not further attenuate the responses to ACh in the RVH state. Values are mean±SEM for 6 dogs.

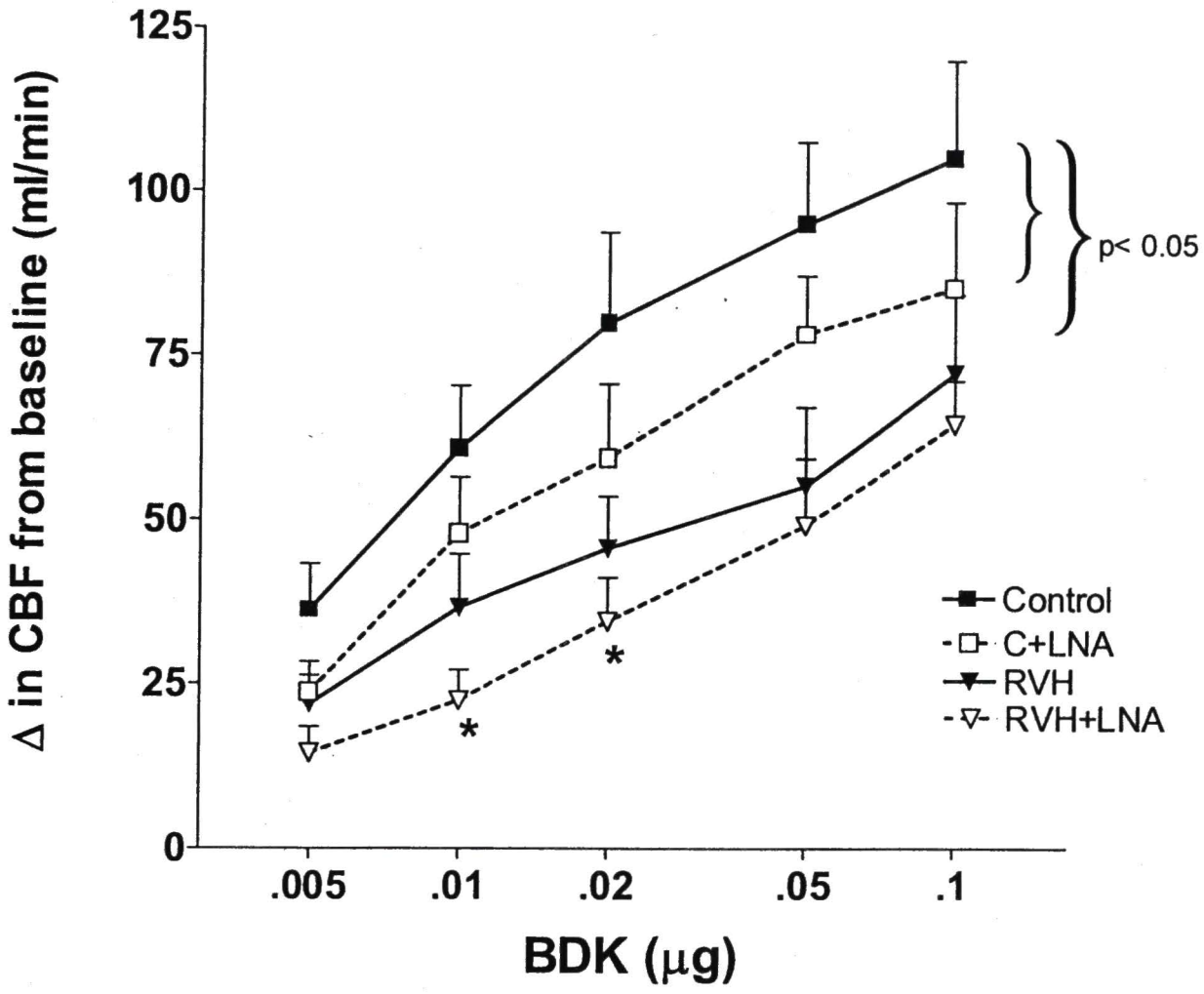

Figure 2-A. Increases in CBF from baseline in response to intracoronary bradykinin (BDK) before and after NO-blockade with Nω-nitro-L-arginine (LNA) in the normotensive (control) and renovascular hypertensive (RVH) states. In the control condition, LNA significantly reduced ($P<0.05$) the CBF response at all doses (Control vs. C+LNA). RVH resulted in a significantly ($P<0.05$) reduced vasodilation to BDK at all doses except 0.005μg, when compared to Control. LNA in the RVH state did further attenuate the vasodilation to BDK 0.01 and 0.02μg doses ($P<0.05$). Values are mean±SEM for 6 dogs. *$P<0.05$ vs RVH.

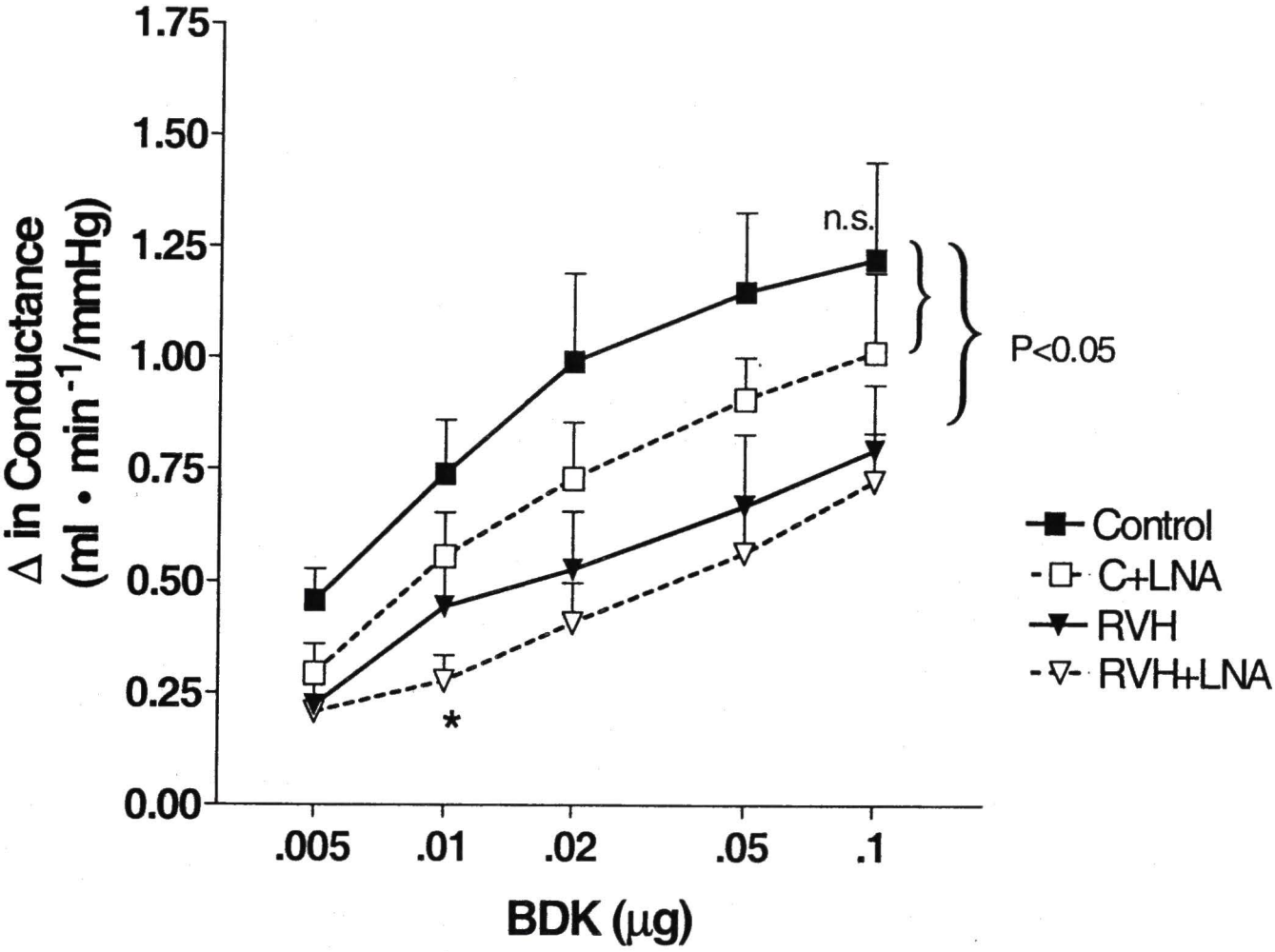

Figure 2-B. Increases in coronary conductance from baseline in response to intracoronary bradykinin (BDK) before and after NO-blockade with Nω-nitro-L-arginine (LNA) in the normotensive (control) and renovascular hypertensive (RVH) states. In control dogs, LNA significantly reduced ($P<0.05$) the CBF response at all doses (Control vs. C+LNA). RVH significantly ($P<0.05$) reduced vasodilation to BDK at all doses, when compared to Control. LNA in the RVH state did further attenuate the vasodilation to BDK 0.01 μg ($P<0.05$). Values are mean±SEM for 6 dogs. *$P<0.05$ vs RVH.

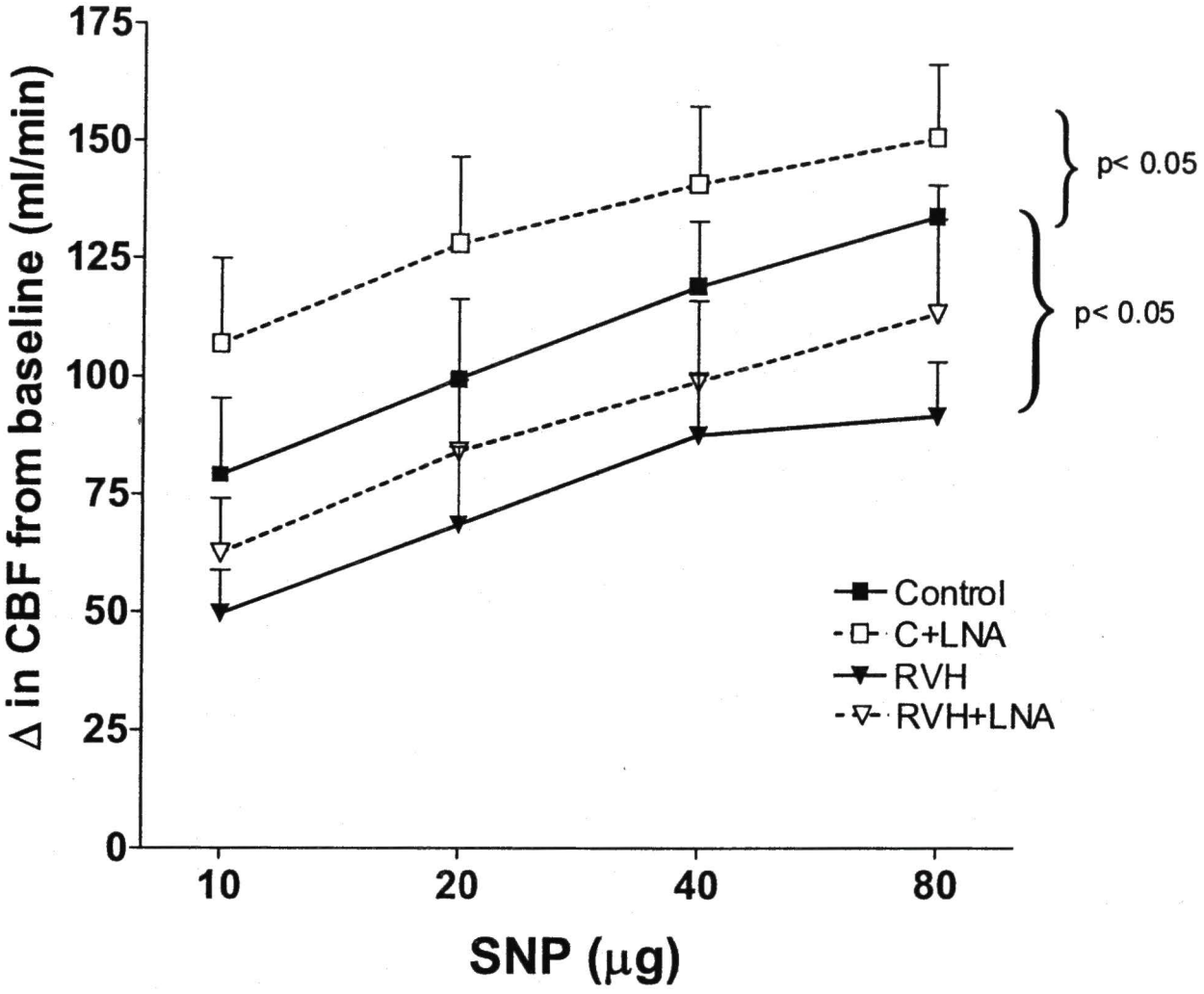

Figure 3-A. Increases in CBF from baseline in response to intracoronary sodium nitroprusside (SNP) before and after NO-blockade with Nω-nitro-L-arginine (LNA) in the normotensive (control) and renovascular hypertensive (RVH) states. In the control condition, LNA significantly enhanced ($P<0.05$) the CBF response at all doses (Control vs. C+LNA). RVH resulted in a significantly ($P<0.05$) reduced vasodilation to SNP at all doses, when compared to Control. Values are mean±SEM for 6 dogs.

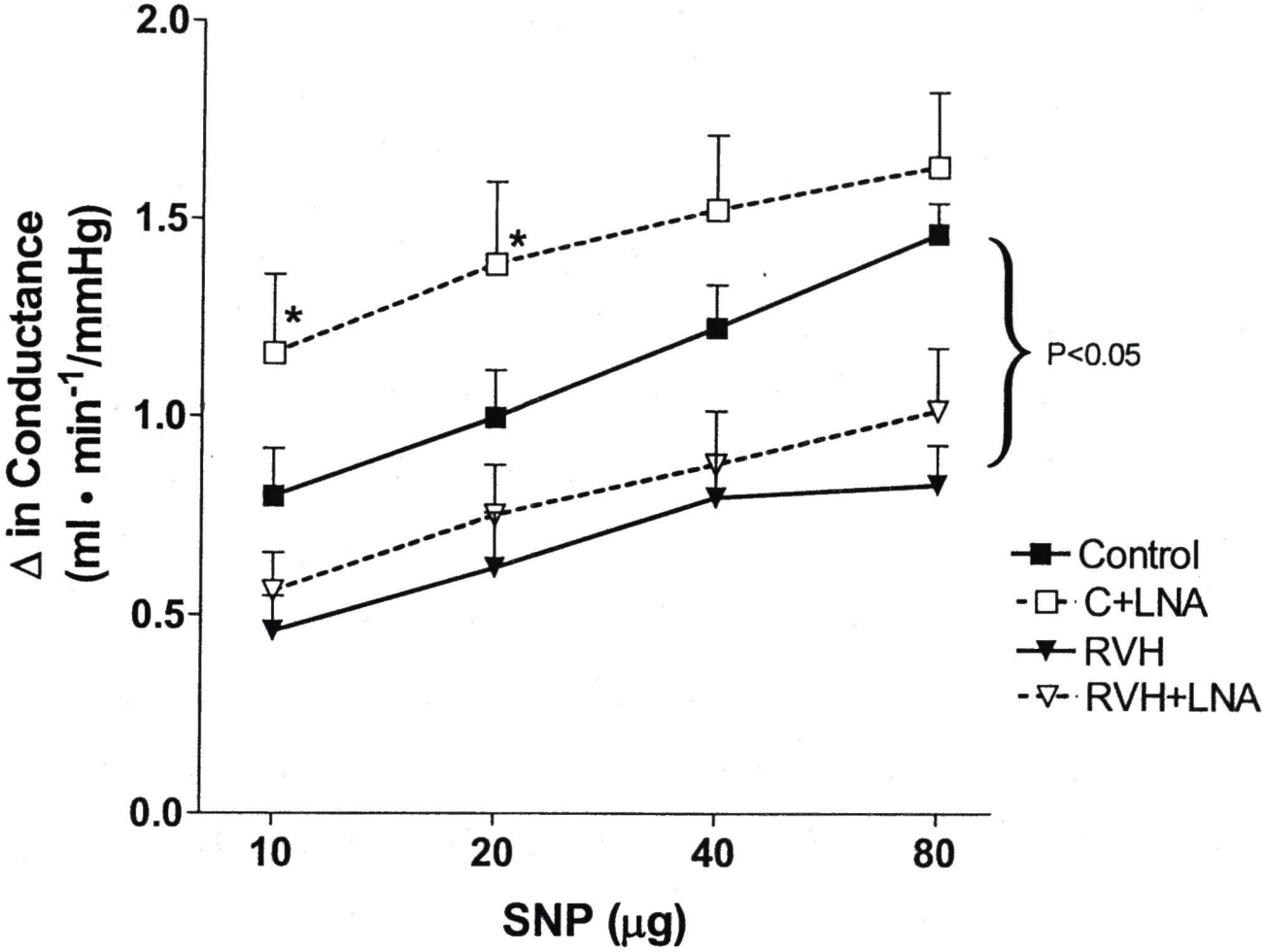

Figure 3-B. Increases in coronary conductance from baseline in response to intracoronary sodium nitroprusside (SNP) before and after NO-blockade with Nω-nitro-L-arginine (LNA) in the normotensive (control) and renovascular hypertensive (RVH) states. In control dogs, LNA significantly enhanced ($P<0.05$) the CBF response at 10 and 20 μg (Control vs. C+LNA). RVH resulted in a significantly ($P<0.05$) reduced vasodilation to SNP at all doses, when compared to Control. Values are mean±SEM for 6 dogs. * $P<0.05$

PREFACE TO CHAPTER III

The previous study examined the hypothesis that the reduced coronary vasodilatory capacity often observed in hypertension is due to an impairment of the endothelial-dependent vasodilatory mechanisms. Specifically, this study investigated the possibility that endothelial NO-mediated coronary vasodilation was altered early in the pathogenesis of renovascular hypertension in conscious dogs. We have found that after only two weeks of hypertension, the coronary vasoreactivity to both endothelial-independent and dependent vasodilator agents is reduced. It appears that in our studies an increased inactivation of NO and/or a decreased sensitivity of the vascular smooth muscle may cause this dysfunction. However, our data indicate that it is likely that this is not the only mechanism responsible for the reduced coronary vasoreactivity. The previous study has demonstrated that after renovascular hypertension the coronary vasculature retains some ability to produce NO. Additionally, it was shown that impairments to endothelial-dependent vasodilators were not manifested in the same manner. Therefore, it appears that the alterations in coronary vasoreactivity during hypertension are not due to a universal endothelial dysfunction, rather it is possible that specific changes occur in some, but not all, pathways involved in endothelial-dependent coronary vasodilation.

The following chapter presents studies that examined the hypothesis that the reduced coronary vasodilatory capacity after hypertension is due to a decreased β-adrenergic receptor mediated vasodilation. We investigated this in conscious dogs before and two weeks after induction of renovascular hypertension.

CHAPTER III

CORONARY β_2-ADRENERGIC VASODILATION IS SELECTIVELY REDUCED EARLY IN RENOVASCULAR HYPERTENSIVE CONSCIOUS DOGS

Geoffrey P. Kline and Patricia A. Gwirtz

ABSTRACT

Hypertension is often associated with impaired coronary vasodilation. This study was performed to investigate the hypothesis that β-adrenergic mediated coronary vasodilation is involved in the impairment of coronary vasodilation after renovascular hypertension (RVH). Specifically, we examined the effects of RVH on β_1-receptor and β_2-receptor mediated coronary vasodilation in conscious dogs. Six dogs were chronically instrumented to measure left ventricular pressure (LVP), $+dP/dt_{max}$, heart rate (HR), mean aortic pressure (AOP), circumflex blood flow (CBF), and cardiac output (CO). The increases in CBF after intracoronary (i.c.) bolus injections of graded doses of norepinephrine (NE) (0.05 to 1.0 μg) and isoproterenol (ISO) (0.001 to 0.1 μg) were measured. In the normotensive state, NE (0.05 μg) increased CBF 37±10 ml/min and ISO (0.01 μg) increased CBF 41±9 ml/min. The increases in CBF to NE and ISO were significantly reduced after RVH ($P<0.05$). After β_1-blockade with atenolol (1 mg, i.c.), ISO (0.01 μg) increased CBF 32±6 ml/min ($P<0.05$ vs. control). The increase in CBF to ISO (0.01 μg) after atenolol was significantly reduced after RVH ($P<0.05$). Bolus injections of the selective β_2-agonist, terbutaline (TRB) were made (1-10 μg). TRB (5 μg) increased CBF 79±13 ml/min, after RVH these responses were reduced ($P<0.05$). After β_2-blockade with ICI-118,551 (1 mg, i.c.), ISO (0.01 μg) increased CBF 9±4 ml/min; this response was not reduced after RVH. These data indicate that β-adrenergic coronary vasodilation is impaired after RVH. Specifically, after RVH β_2-adrenoceptor

mediated vasodilation is substantially reduced, while β_1-adrenoceptor mediated vasodilation appears to be preserved.

Keywords: Coronary circulation, renovascular hypertension, β-adrenergic

INTRODUCTION

Increases in coronary vascular resistance and reduced vasodilatory capacity are often associated with hypertension, even in the absence of left ventricular hypertrophy. [1-4] The mechanisms responsible for impaired coronary vasodilatory capacity in systemic hypertension are unclear, but may include altered adrenergic, neurohumoral, or vascular endothelial mechanisms.

While studies have recently concentrated on impairment of endothelia-dependent vasodilation during conditions of hypertension, few studies have investigated the possibility that β-adrenergic coronary vasodilation is impaired in hypertension. β-receptors are located on vascular smooth muscle and cardiac myocytes. Stimulation of coronary vascular β_2-receptors results in vasodilation.[5,6] Stimulation of cardiac β_1-receptors result in an increase in heart rate and contractility, which lead to a secondary metabolic vasodilation. There is growing evidence for the existence of β_2-adrenergic receptors on the endothelium in the canine coronary circulation.[5-8] Several studies have shown that β_2-receptor mediated vasodilation in coronary resistance vessels is actually

dependent on endothelial nitric oxide (NO) formation.[5,6] Studies in patients[2,9,10] and animal models[11,12] of hypertension suggest that a dysfunctional endothelium, resulting in abnormal endothelial-dependent vasodilation, contributes to the impaired coronary dilatory capacity. Endothelium-dependent relaxation appears to be depressed in the aorta and large arteries of various models of hypertension,[2,11,13-15] while endothelial-independent vasodilation is preserved.[2,9,16,17] In light of these data, it is therefore possible that β-adrenergic mediated coronary vasodilation is also impaired after hypertension.

The present studies were conducted to test the hypothesis that the reduced coronary vasodilatory capacity in renovascular hypertension (RVH) is partially mediated by impaired β_2-adrenergic vasodilation. We also examined the function of coronary β_1-adrenergic vasodilation before and after RVH, to determine the integrity of this system after RVH. These studies were conducted in chronically instrumented, conscious dogs before and after induction of RVH.

MATERIAL AND METHODS

Surgical Preparation

Six (6) healthy, mongrel dogs of either sex (weight range 25-35 kg) were studied. All dogs were premedicated with acepromazine (0.03 mg/kg, s.c.) and anesthetized with thiopental sodium (5 mg/kg, i.v.). The trachea was intubated and anesthesia maintained with isoflurane gas (1-3%) with an equal offset of O_2 (1 L). Using sterile technique, a

thoracotomy was performed through the left fifth intercostal space and the heart instrumented as previously described in detail.[18]

The aorta was exposed and a fluid-filled Tygon catheter was inserted just distal to the aortic arch to monitor aortic pressure (AOP). A Transonic transit time Doppler flow probe (Transonic Systems Inc, Ithaca, NY) was placed around the root of the aorta to measure cardiac output (CO). For the measurement of left ventricular pressure (LVP) a Konigsberg P-6.5 transducer solid state micromanometer (Konigsberg Instruments Inc, Pasadena, CA) and a fluid-filled Tygon catheter were inserted into the left ventricle through a stab-wound in the apex. The Konigsberg pressure transducer was calibrated before implantation and routinely checked and adjusted by simultaneously measuring pressure with the implanted Tygon catheter connected to an external Isotec® pressure transducer, which was calibrated using a mercury manometer. The circumflex artery was dissected free of the surrounding tissue for a distance of approximately 3 cm beginning at the origin of the vessel. A 10 MHz Doppler ultrasonic flow probe (4 mm ID) was positioned around the circumflex artery for measurement of circumflex blood flow velocity (CFV) using a Triton Flowmeter (San Diego, CA). At necropsy, the internal circumference of the vessel under the probe was measured to obtain the vessel cross-sectional area which was used to convert CFV to coronary blood flow (CBF). To check the zero flow reference, a pneumatic occluder was placed around the circumflex artery immediately distal to the Doppler ultrasonic flow probe such that there was no vessel branch between the two. The circumflex artery was cannulated with a heparin-filled

Silastic catheter distal to the occluder for injection of the solutions into the circumflex artery.[19]

After instrumentation was completed, a chest tube was placed in the thoracic cavity to evacuate the pneumothorax and any post-surgical intrathoracic exudate accumulation. All wires and catheters were tunneled subcutaneously to exit between the scapula. Indwelling catheters were flushed daily with heparinized saline to maintain patency. Post-operative analgesics, antibiotics, and antipyretics were given by the veterinarian for 5 days, or as needed. At least 10 to 14 days were allowed to elapse before experiments were begun. During this time the dogs were familiarized to the laboratory setting.

After initial control (normotensive) studies were performed, the dogs were re-anesthetized as described above and a midline laparotomy performed. Both the left and right renal arteries were isolated and 10-MHz Doppler flow probes and saline filled occluders were placed around the vessels. The incision was then closed in layers and occluder and lead wires were tunneled subcutaneously to exit between the scapula. All protocols were reviewed and approved by the institutional Animal Care and Use Committee.

Model of Renovascular Hypertension

Hypertension was induced by the unilateral renal stenosis method (two-kidney, one clip Goldblatt hypertension model) described by Anderson *et al.*[20] This model is more appropriate for the study of renovascular hypertension (RVH) than the one-kidney

models, which are more appropriate for the study of renal parenchymal hypertension[21] After prestenosis, or normotensive, studies were completed one of the renal arteries was stenosed by inflating one of the saline filled occluder until renal flow was reduced by 60%.[22] Verification of stenosis was performed several times daily. Studies were repeated 2 weeks after a stable hypertensive state was achieved. Blood pressure was monitored on a daily basis during developing and stable states of hypertension.

Experimental Protocol

In all experiments, the dogs were trained to lay quietly on the table in a calm, resting manner. Studies were performed only if resting HR was ≤ 90 bpm, to ensure a resting state.

Normotensive Studies. Experiments were performed 2 weeks after initial surgery to examine the coronary vascular reactivity to β-adrenergic stimulation and to determine the relative contributions of the β_1- and β_2-adrenergic receptor subtypes in these responses. After resting control measurements, dose-coronary flow responses to several β-adrenergic agonists were obtained. Bolus intracoronary (i.c.) injections of the endogenous neurotransmitter, norepinephrine (NE) (0.05, .01, 0.3, 0.5, 1.0 μg), the mixed β_1-β_2-adrenergic agonist, isoproterenol (ISO) (0.001, 0.005, 0.01, 0.05, 0.1 μg), and the selective β_2-adrenergic agonist, terbutaline (TRB) (1, 5, 10 μg), were made. During these experiments all hemodynamic variables were continuously recorded and monitored. Drugs and doses were given in a randomized order. Doses were selected to produce dose-dependent increases in CBF with minimal affect on other hemodynamic variables.

All drugs were dissolved in sterile saline and then filtered with sterile Acrodisc (0.2 μm) filters (Gelman Sciences) on the day of the experiment. Volume of the drugs injected were ≤1.0 ml and were followed by a saline flush of 2 ml over 18 seconds. In each dog it was verified that a vehicle flush of this volume and speed did not alter CBF. CBF data reported was measured at the peak response to each drug. CBF was allowed to return to baseline for several minutes before administering the next dose.

On a separate day, we examined the role of β_2-receptors in mediating the vasodilation to ISO by blocking β_1-receptors with the selective β_1 antagonist, atenolol (AT) (1 mg, i.c.). TRB was given to ensure that AT did not significantly block β_2-receptors as well. Dose responses to ISO and TRB were repeated after β_1-adrenergic blockade.

On another day, dose responses to ISO and TRB were repeated after β_2-adrenergic blockade with the selective β_2 antagonist, ICI-118,551 (ICI) (1 mg, i.c.). This study was performed to isolate the relative contribution of β_1-adrenergic receptor activation in the CBF response to i.c. ISO. TRB was given after ICI to ensure that β_2 responses were effectively blocked.

Hypertensive Studies After normotensive studies were completed, all dogs were made hypertensive using the method described above, thus each dog served as its own control. Two weeks into the hypertensive state, the protocols described above were repeated. Dose responses to NE, ISO, and TRB were performed in the same manner as those in the

normotensive state. Likewise, the dose-responses to ISO and TRB were repeated after β_1 blockade with AT and β_2 blockade with ICI, on different days.

Data Collection and Analysis

Signals were simultaneously recorded on a Coulbourn 8-channel paper chart recorder (Allentown, PA) and a 15-channel Vetter Model 4000A PCM recording adaptor (A.R. Vetter, Rebersburg, PA) for digital recording with a Vetter-modified Model 500 VCR . Signals were also sent to an IBM compatible computer using a PowerLab/800 (ADInstruments) system. All data were recorded in real-time and later analyzed using the computer analysis program Chart (v3.4) for Windows (ADInstruments). The following data were analyzed from the recorded variables: left ventricular systolic pressure (LVSP), maximum rate of left ventricular pressure development ($+dP/dt_{max}$), heart rate (HR), mean arterial pressure (AOP), CO, and CFV.

Data are reported as mean ± standard error of the mean (SEM), and differences between means were considered statistically significant if the probability of their occurring by chance was less than 5% ($p < 0.05$). Multiple simultaneous comparisons of baseline data in control conditions, after LNA, after RVH, and after RVH+LNA were made using an analysis of variance for repeated measures followed by Student-Newman-Keuls test to isolate specific contrasts. Comparisons of drug responses before and after the selective β-blockers before and after RVH were made using paired *t* tests.

RESULTS

Baseline Hemodynamics Before and After β-blockades

RVH resulted in a significant increase in mean LVSP and AOP and significant decreases in HR, CO, and CBF (see Table 1). There was no change in resting $+dP/dt_{max}$. In order to examine the function of β_1 and β_2 receptors selectively, dose-coronary flow responses to ISO after selective β_1 and β_2-adrenergic blockade were made. The hemodynamic effects of i.c. AT and ICI, in the resting state are also shown in Table 1. In the normotensive and RVH states, β_1-blockade with AT did not significantly alter any of the measured hemodynamic variables, when compared to respective controls. However, in both normotensive and RVH dogs, β_2-receptor blockade with ICI significantly reduced resting CBF, when compared to respective controls. ICI did not affect any other hemodynamic variables.

Responses to Norepinephrine

All dose-response data shown in Figures 1-4 are reported as change in CBF from the baseline levels presented in Table 1. Dose-response curves for NE before and after RVH are shown in Figure 1, panel A. Graded doses of i.c. NE caused dose-dependent increases in CBF. In the normotensive state, 0.05, 0.1, 0.3, 0.5, and 1.0 μg doses of NE increased CBF above baseline by 5, 25, 48, 58, 97 percent, respectively. When compared to the normotensive state, the vasodilatory responses to i.c. 0.3, 0.5, and 1.0 μg of NE were significantly reduced after RVH.

Responses to Isoproterenol

Dose-response curves for ISO before and after RVH are shown in Figure 1, panel B. Bolus i.c. injections of ISO resulted in dose-dependent increases in CBF. In the normotensive state, 0.001, 0.005, 0.01, 0.05, and 0.1 μg doses of ISO increased CBF above baseline by 10, 42, 65, 127, and 142 percent, respectively. After 2 weeks of RVH the vasodilation in response to the three highest doses of ISO was significantly reduced. These data suggest that after RVH β-adrenergic coronary vasodilation is impaired.

Responses after selective β_1 and β_2 blockades

Studies were also performed to examine the effect of RVH on selective β_2-adrenoceptor mediated coronary vasodilation. This was accomplished by administering a selective β_2-agonist, TRB, i.c., and by blocking β_1-adrenergic receptors to uncover the β_2 activity in the coronary vasodilatory response to i.c. ISO. Dose-response curves for i.c. TRB before and after RVH are shown in Figure 2, panel A. Bolus injections of TRB, i.c., resulted in dose-dependent increases in CBF. In the normotensive state the 1, 5, and 10 μg doses of TRB increased CBF above baseline by 93, 124, and 146 percent, respectively. After RVH, TRB-induced coronary vasodilation was significantly reduced at all doses. β_1-blockade with AT (1 mg, i.c.) significantly reduced the vasodilation to 0.1 μg ISO, in both the normotensive and RVH states (Figure 3, panel A). These data indicate that AT effectively blocked coronary β_1-receptors. β_1-blockade did not affect the vasodilation to TRB in either the normotensive or RVH states (Figure 3, panel C). Thus, AT resulted in a significant blockade of β_1-adrenoceptors and had no effect on β_2-

adrenoceptor mediated coronary vasodilation. ISO (0.01 μg) given after AT in the normotensive state caused CBF to increase 31.7±6.2 ml/min, in contrast, after RVH this response was significantly ($P<0.05$) reduced to 10.9±3.4 ml/min. Thus, these experiments demonstrate that: 1) β_1-blockade with AT effectively blocked β_1-adrenoceptors but not β_2-adrenoceptors, and 2) β_2-adrenergic coronary vasodilation is impaired after RVH.

Studies were also performed to examine the effect of RVH on β_1-adrenoceptor mediated coronary vasodilation. This was done by examining the effects of β_2-adrenergic blockade on the coronary vascular response to i.c. ISO, thus revealing β_1 activity. In both the normotensive and RVH states, β_2-blockade with ICI resulted in a near complete elimination of coronary vasodilation to i.c. TRB (Figure 3, panel B). Dose-response curves for i.c. ISO during β_2-blockade before and after RVH are shown in Figure 2, panel B. Bolus injections of ISO after β_2-blockade resulted in dose-dependent increases in CBF. In the normotensive state 0.001, 0.005, 0.01, 0.05, and 0.1 μg doses of ISO, during β_2-blockade, increased CBF above baseline by 7, 8, 17, 35, and 44 percent, respectively. In contrast, RVH did not alter the vasodilatory response to i.c. ISO during β_2-blockade. Thus, these data demonstrate that our β_2-blockade was nearly complete and that after RVH coronary β_1-adrenoceptor responses are preserved.

DISCUSSION

This is the first study to examine the coronary responsivity to β-adrenergic stimulation after short-term RVH in a conscious animal. The major findings of this study are that β_2-adrenergic coronary vasodilation is impaired following two weeks of RVH, while β_1-adrenergic mediated coronary vasodilation appears to be preserved.

RVH is a clinically prominent form of secondary hypertension which is associated with elevated levels of circulating angiotensin II. During the early phase of two kidney-one clip RVH, the pathogenesis of the disease has been attributed to a combination of elevated angiotensin II, altered plasma volume, changes in renal function, and increased renal nerve activity.[20,23] In the present study, unilateral renal artery stenosis caused a significant 20 mmHg increase in resting mean AOP and LVSP. Resting CO, HR, and CBF were simultaneously reduced.

Studies were first performed to examine the effect of RVH on coronary responsivity to the endogenous neurotransmitter, NE, and the nonselective β-adrenergic agonist, ISO. In these studies we have shown that there is an early impairment in the coronary vasodilation to these vasoactive agents after RVH. These changes in coronary vasoreactivity may be due to several reasons. Many factors contribute to the increased CBF induced by i.c. NE. First, NE directly stimulates coronary β-receptors on the vascular smooth muscle to cause vasodilation. NE also causes metabolic vasodilation secondary to increased work in the adrenergically stimulated myocardium.[24,25] In addition, NE-induced coronary vasodilation has been found to be partially dependent on

flow-dependent increases in endothelial NO formation.[26] Studies have also found evidence of β_2-adrenoceptors on the canine coronary vascular endothelium.[5,7,8,27] Several of these studies have shown that β_2-receptor mediated vasodilation in coronary resistance vessels is dependent on endothelial NO formation.[5,27] However, in the large coronary conductance vessels, β_2-adrenergic receptors do not appear to be functionally coupled with NO synthesis. Rather, it is thought that β_2-receptor stimulation results in large vessel dilation that is largely a flow-dependent phenomenon.[7,8] Therefore, it is likely that NE induces coronary vasodilation by stimulating β_1-receptors on the vascular smooth muscle and myocardium in addition to the endothelial β_2-receptors (in small resistance vessels). NE also stimulates α- adrenergic receptors in the coronary vasculature, so it is possible that the observed decrease in NE-induced vasodilation was due to an enhanced α-mediated vasoconstriction in response to RVH, rather than a decreased β-mediated vasodilation. However, ISO only activates β-receptors and these responses were reduced after RVH. These data, therefore, suggest that after RVH β-mediated vasodilation is impaired. It is likely that at least part of the decreased coronary vasodilation to NE was due to an impaired β-mediated vasodilation. It is possible that after RVH the vasodilation to i.c. NE is impaired by both a decreased β-receptor mediated vasodilation and an augmented α-receptor mediated vasoconstriction.[28]

Our data indicate that early in the RVH disease process, β_2-adrenergic mediated coronary vasodilation is impaired. This was demonstrated with both decreased responses to the selective β_2-receptor agonist TRB and to ISO after atenolol. Our data also suggest

that coronary β_1-adrenergic mediated vasodilation is preserved in the RVH state. This was demonstrated by the preserved vasodilatory responses to ISO after β_2-blockade with ICI. Both of these findings are supported by previous studies by others using a variety of animal models of hypertension. Shannon *et al* reported that after perinephritic hypertension, the inotropic response to β-adrenergic stimulation was preserved in conscious dogs.[29] Studies using hypertensive rats have shown that β-adrenergic vasodilation is impaired in the aorta and femoral and mesenteric arteries.[30,31] However, Fuchs *et al* found that ISO induced vasodilation of isolated coronaries from rats with genetic hypertension was not impaired.[16] In this study, they only examined the responsivity of arteries (200-300 μm). Larger coronary vessels have a much greater distribution of β_1- than β_2- adrenergic receptors.[32-34] However, the small coronary arterioles (20-70 μm) have a much greater proportion of β_2- than β_1-adrenoceptors.[35] Therefore, in the Fuchs *et al* study, it is possible that the preserved ISO dilation was due to mainly an activation of smooth muscle β_1-receptors. This would then tend to support our findings of a preserved β_1-mediated coronary vasodilation after RVH.

Our finding that β_2-mediated coronary vasodilation, but not β_1-mediated, is impaired after RVH may be explained by the possibility of a dysfunctional vascular endothelium after RVH. Studies in a variety of tissues and animal species have demonstrated a vascular endothelial dysfunction after hypertension that is likely due to generalized defect in the endothelial production and release of NO.[2,11,13,14] This fact along with the evidence that β_2-adrenoceptor mediated coronary vasodilation is linked to

endothelial NO production makes it likely that the decreased vasodilation to β_2-receptor stimulation that we observed in the present study is due to coronary vascular endothelial dysfunction. On the contrary, it has been shown that β_1-mediated coronary vasodilation in conscious dogs is insensitive to blockade of NO synthesis.[8] This indicates that β_1-mediated coronary vasodilation is due to either direct activation of vascular smooth muscle or metabolic vasodilation secondary to myocardial stimulation. Our data indicate that these mechanisms mediating β_1 coronary vasodilation are maintained during RVH.

We explored the possibility that the selective β_1-blockade with AT also partially blocked β_2-receptors as well. We found no evidence of this as demonstrated by the lack of an effect of AT on the responses to TRB, a selective β_2-agonist. The dose of AT we used in this study has been previously shown to effectively block coronary β_1-adrenoceptors.[36] Effectiveness of this blockade was also verified in the present study by demonstrating that AT (1 mg, i.c.) significantly reduced ISO-induced vasodilation. We were unable to directly determine if the selective β_2-blockade with ICI also resulted in a significant blockade of β_1-receptors. However, the responses to TRB after ICI were almost completely eliminated, while ISO after ICI retained a significant vasodilation. Therefore, we concluded that both the β_1- and β_2-blockades we used were specific and effective. It should be noted that our β_2-block with ICI was not an absolute blockade; when verified with TRB the responses were blocked 85-90%. Therefore, it is possible that the small responses we measured to ISO after ICI were partially due to residual β_2 stimulation.

We have shown in these experiments that both NE- and ISO-induced coronary vasodilation are reduced after two weeks of RVH. These studies have also demonstrated that this impairment appears to be selective to β_2-receptor mediated vasodilation, leaving the β_1-mediated vasodilation intact. Figure 4 illustrates the combined findings of this study. As illustrated in Figure 4, it appears that the relative contributions of the β_1- and β_2-receptors in ISO-induced coronary vasodilation are additive in the normotensive and RVH states. As indicated, the impaired vasodilation to ISO after RVH is due to a selective impairment of the β_2-mediated vasodilatory mechanism.

Stimulation of myocardial β_1-receptors results in increased myocardial oxygen consumption which then creates a secondary local metabolic feedback vasodilation.[24] The imbalance between metabolic demand and oxygen delivery provides an error signal for the metabolic feedback system to operate and cause local vasodilation.[24] A study conducted by Miyashiro *et al* has also found evidence for direct feedforward β-receptor mediated coronary vasodilation.[25] In their study they found evidence of β-receptor mediated vasodilation independent of chronotropic and inotropic effects. It is thought that this feedforward vasodilation could allow a rapid increase in oxygen supply while minimizing the imbalance between supply and demand, thereby decreasing the error signal generated.[25] In the present study, we found that β_1-mediated coronary vasodilation is preserved in RVH, thus it is possible that the described feedback vasodilatory control of coronary blood flow is unaffected by hypertension. However, since β_2-receptors are thought to cause vasodilation by stimulating NO production from

the endothelium, without stimulating the myocardium, it is likely that this mechanism is involved in the described feedforward control of coronary blood flow. In this study we have described an impairment in the β_2-mediated coronary vasodilation, therefore, it is possible that a proposed β-adrenergic feedforward control of coronary blood flow is impaired in hypertension. This loss or impairment of the feedforward control mechanism may impair or alter cardiac function in the hypertensive state and explain some of the limited cardiac function and exercise tolerance clinically associated with hypertension.

In summary, we have found that after two weeks of RVH, conscious dogs exhibit an impaired vasodilation to NE and ISO. This appears to be due to a selective impairment of β_2-mediated, but not β_1, coronary vasodilation. It is possible that the observed dysfunction in endothelial-dependent vasodilation found in clinical and experimental forms of hypertension may be related.

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Table 1. Baseline hemodynamic values before and after selective β-blockades in the Control and RVH conditions

	Normotensive State			Hypertensive State		
	Baseline	β_1-Blockade	β_2-Blockade	Baseline	β_1-Blockade	β_2-Blockade
LVSP (mm Hg)	121±5	120±6	123±4	148±6†	149±5†	150±7†
$+dP/dt_{max}$ (mm Hg/sec)	2861±223	2506±318	2389±264	3532±560	3459±461	3642±355
Mean AOP (mm Hg)	93±2	93±3	94±4	113±6†	114±3†	113±7*
CO (L/min)	4.1±0.5	4.0±0.7	4.1±0.8	3.6±0.4*	3.6±0.5*	3.4±0.4*
HR (bpm)	78±2	77±5	75±4	64±4*	65±5*	62±5*
CBF (ml/min)	63.4±5.0	68.2±4.6	52.6±6.3‡	52.5±5.7*	45.2±4.2*	42.0±5.2‡

Abbreviations: LVSP indicates left ventricular systolic pressure; $+dP/dt_{max}$, maximum rate of left ventricular pressure generation; AOP, aortic pressure; CO, cardiac output; HR, heart rate; and CBF, coronary blood flow. Values are ±SEM; n=6. β_1-blockade was induced by intracoronary atenolol (1 mg). β_2-blockade was induced by intracoronary ICI118551 (1 mg).

*P<0.05 compared with normotensive. †P<0.01 compared with normotensive. ‡P<0.05 compared with respective baseline

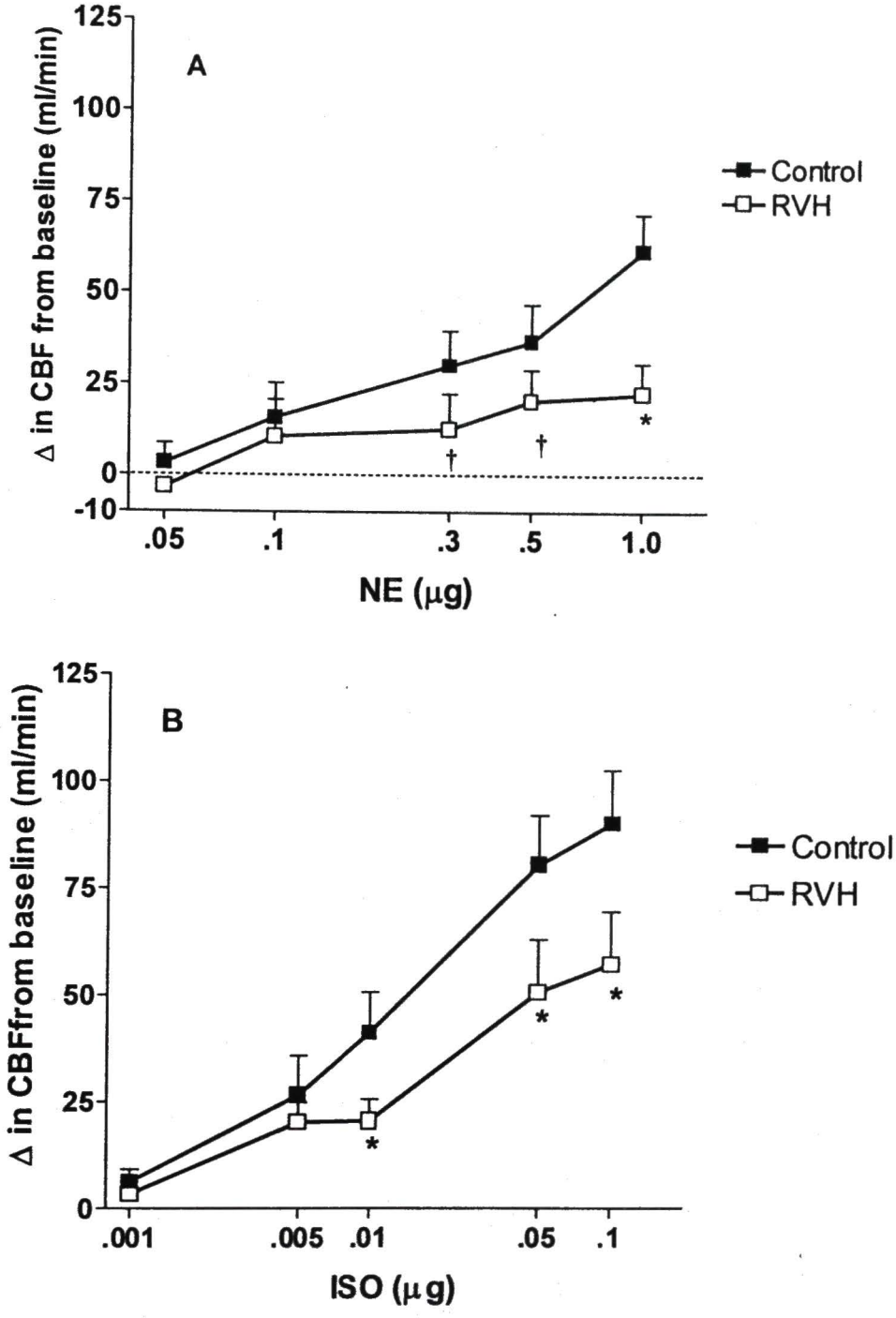


Figure 1. Dose-response curves for intracoronary injections of norepinephrine (NE) and isoproterenol (ISO) before and after RVH. *Panel A,* RVH significantly reduced the vasodilation to NE at the 0.3, 0.5, and 1.0 μg doses. *Panel B,* RVH significantly reduced the vasodilation to ISO at the 0.01, 0.05, and 0.1 μg doses. *P<0.05, †P<0.01.

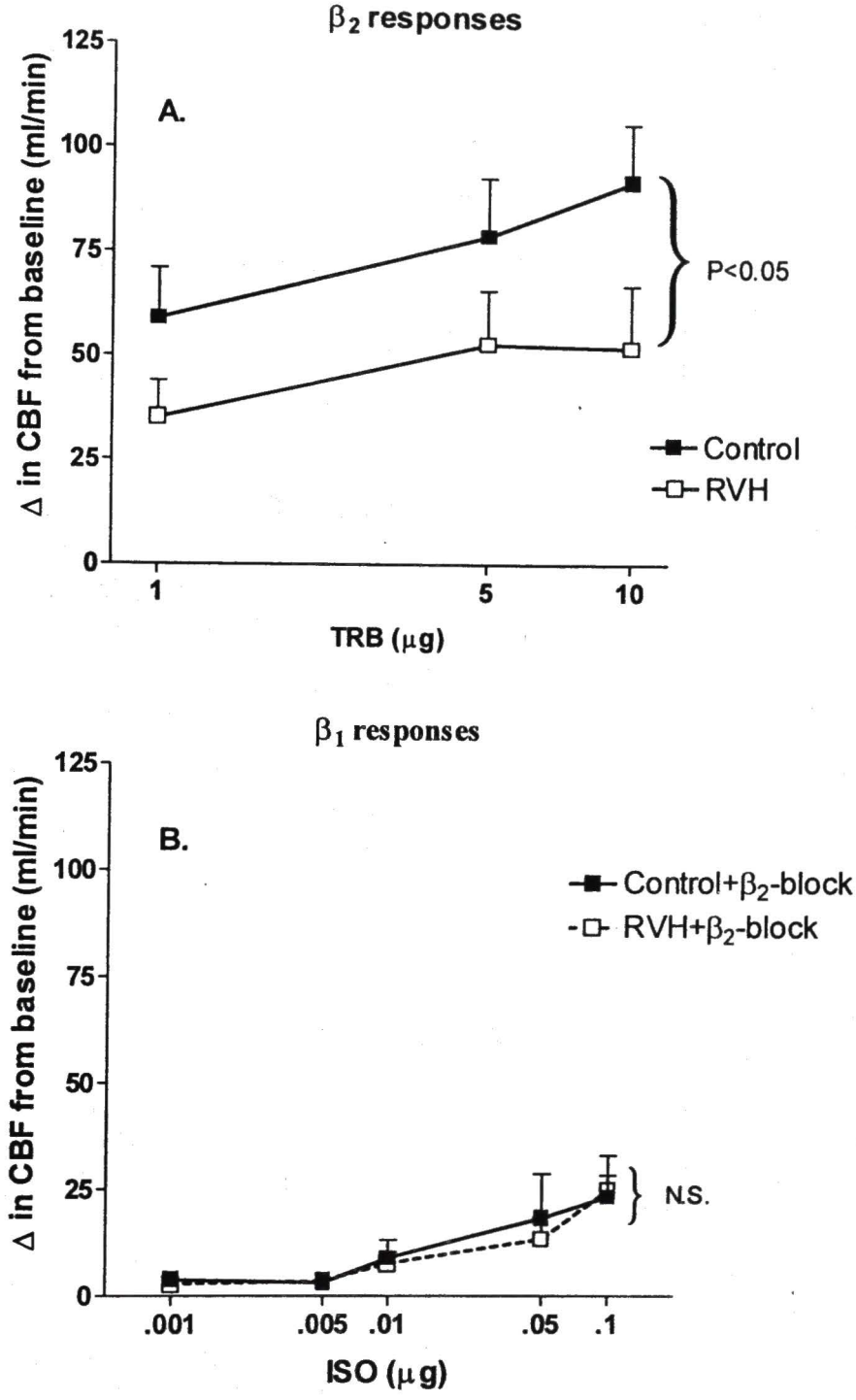
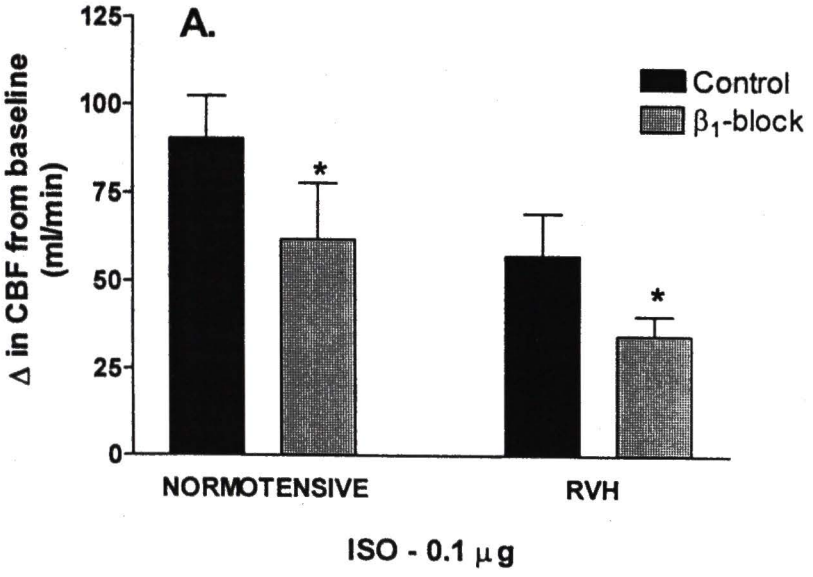
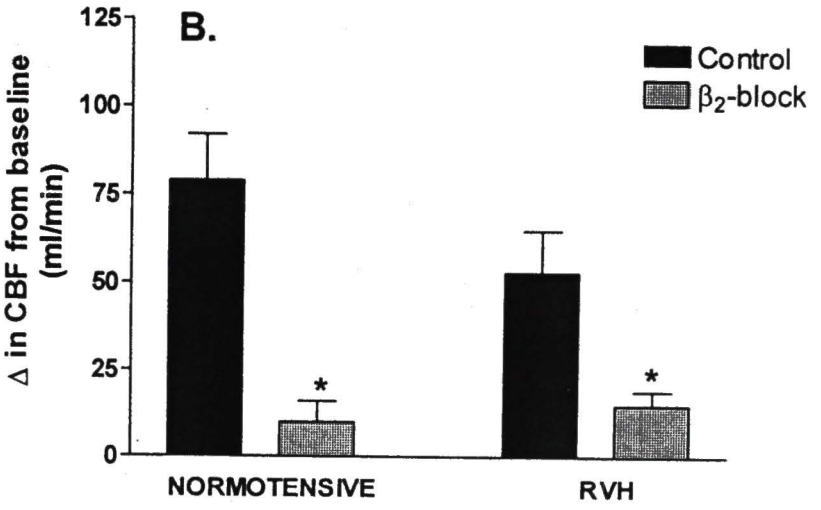


Figure 2. Graphs of dose-response curves for selective β-adrenergic stimulation. *Panel A, β_2 stimulation:* Responses to intracoronary terbutaline (TRB) before and after RVH. *Panel B, β_1 stimulation*: Responses to intracoronary isoproterenol (ISO) after β_2-blockade with ICI-118,551 (1 mg, i.c.), before and after RVH.





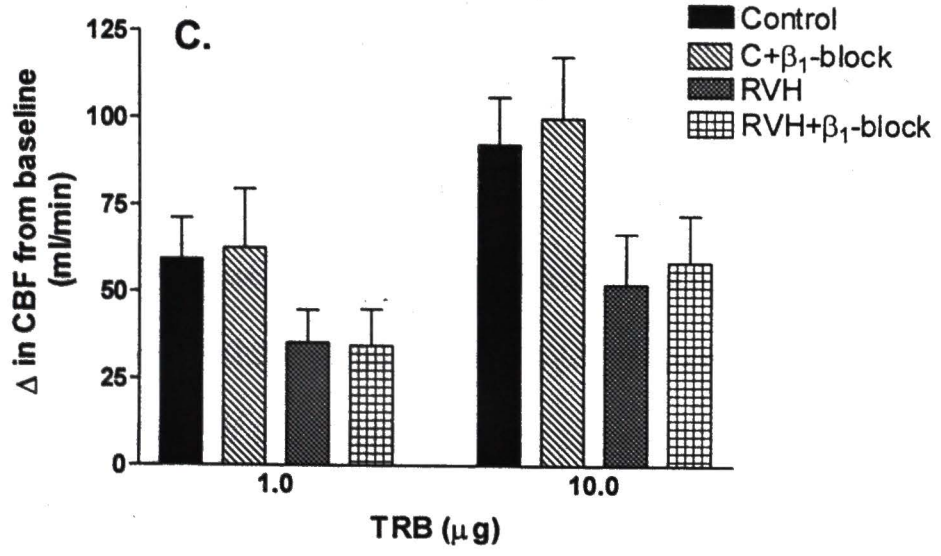


Figure 3. Graphs illustrating effectiveness of β-blockades. *Panel A,* β_1-blockade with intracoronary atenolol (1 mg) resulted in significant decreases in the vasodilation to isoproterenol (ISO) in both normotension and RVH. *Panel B,* β_2-blockade with intracoronary ICI-118,551 (1 mg) resulted in an almost complete block of the response to terbutaline (TRB), both before and after RVH. *Panel C,* β_1-blockade had no effect on β_2 responses, before or after RVH.

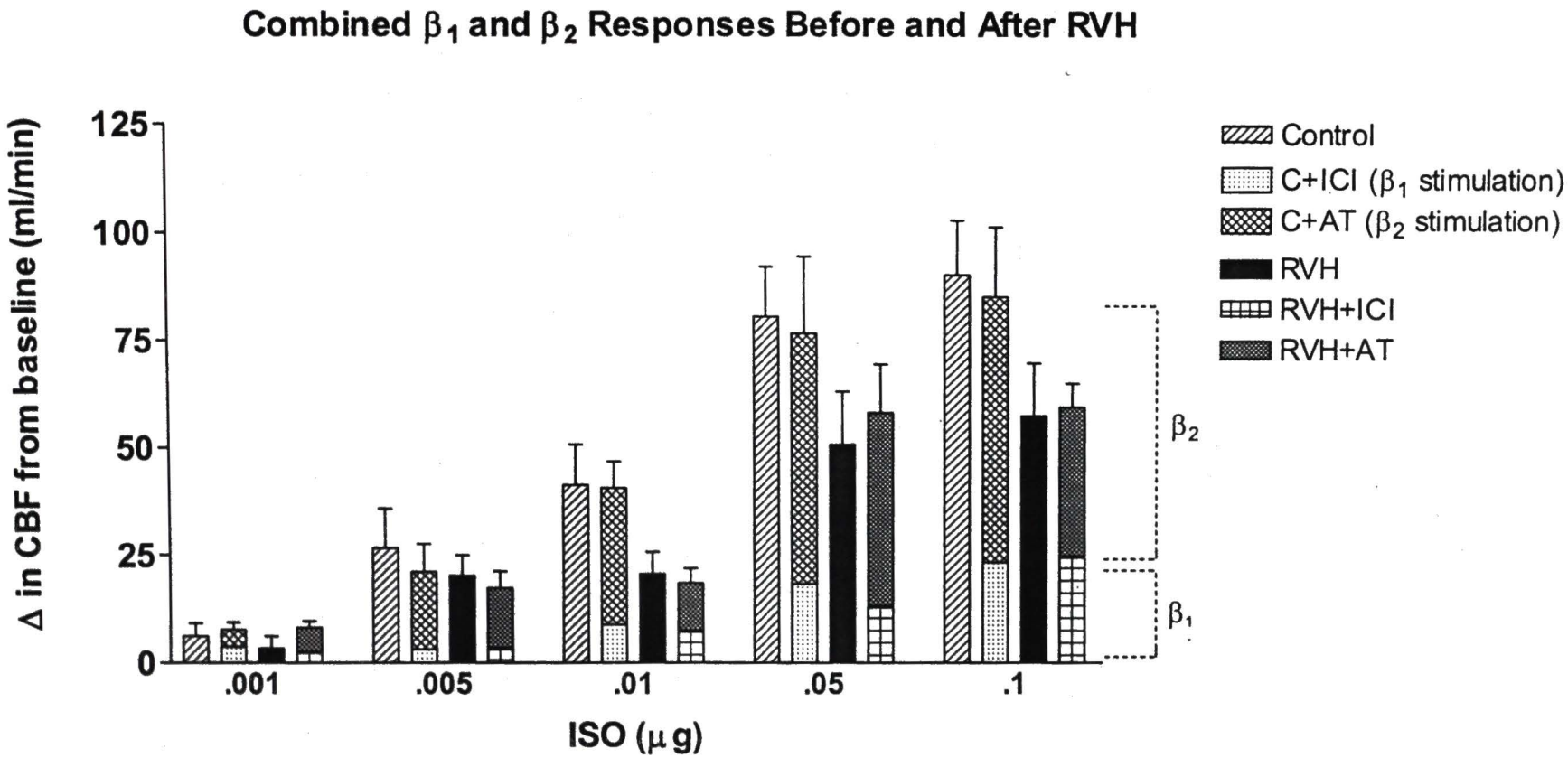


Figure 4. Graph illustrating the relative roles of β_1- and β_2-receptors in the coronary vasodilation to intracoronary (i.c.) isoproterenol (ISO) before and after RVH. B_1-blockade was induced with atenolol (AT) (1 mg, i.c.). β_2-blockade was induced with ICI-118,551 (ICI) (1 mg, i.c.). RVH resulted in a selective impairment of the β_2-mediated coronary vasodilation.

CHAPTER IV

CONCLUSIONS

These studies are the first to describe an early impairment in endothelial-dependent and β_2-adrenoceptor mediated coronary vasodilation in the conscious dog with renovascular hypertension. These studies indicate that the regulation of coronary blood flow is dysfunctional in renovascular hypertension and that these impairments occur before significant vascular or myocardial structural changes are observed. It is therefore possible that these impairments manifest themselves early in patients with undiagnosed hypertension and cause alterations in control of coronary blood flow before clinical symptoms are noticed. These studies provide information lacking in the literature concerning the existence and nature of specific alterations in endothelial and adrenergic control of coronary blood flow. In addition, a major strength of these studies was the ability to determine individual changes in vasoreactivity by using the same dogs before and after induction of renovascular hypertension.

In summary, the major findings of this work were as follows:

1) Renovascular hypertension resulted in significant increases in resting mean arterial and left ventricular systolic blood pressures, while significantly reducing resting heart rate, cardiac output, and coronary blood flow.
2) Renovascular hypertension resulted in a reduced coronary vasodilation to the endothelial-dependent dilators acetylcholine and bradykinin.

3) Nitric oxide was not involved in the acetylcholine-induced coronary vasodilation after renovascular hypertension. However, at lower doses, nitric oxide was involved in the bradykinin-induced coronary vasodilation after renovascular hypertension. Therefore, the endothelium retains some ability to produce nitric oxide after short-term renovascular hypertension.

4) Renovascular hypertension resulted in a reduced coronary vasodilation to the endothelial-independent dilator sodium nitroprusside (an exogenous nitric oxide donor). This suggests that renovascular hypertension results in an increased inactivation of nitric oxide and/or decreased vascular smooth muscle responsivity to nitric oxide.

5) Renovascular hypertension resulted in a reduced coronary vasodilation to norepinephrine and isoproterenol.

6) Renovascular hypertension resulted in a selective impairment of β_2-adrenergic receptor mediated coronary vasodilation. β_1-adrenergic mediated coronary vasodilation appears to be preserved after hypertension.

CHAPTER V

RECOMMENDATIONS FOR FURTHER STUDY

The results presented here answer many questions concerning the impact of hypertension on the control of coronary blood flow. However, it also brings to light many new areas that need to be studied. Some areas that could further clarify the changes we noted could involve examining specific second messenger systems involved in the signal transduction of endothelium-dependent vasodilation to determine if there are dysfunctions in specific pathways, but not in others. The role of the free radicals in these observations should also be determined.

Studies using isolated vessels from RVH dogs should be conducted to examine the vasoreactivity to the same drugs we have used in this study. This approach will allow the study of coronary reactivity without the confounding effects of the intact cardiovascular system. These studies may also allow the more selective inhibition of various steps in the signal transduction pathways activated by the drugs we have administered, thus giving more insight as to where specific changes may be occurring.

To study the role of free radicals in the alterations we have observed, the dose-responses we have conducted should be repeated after i.c. administration of free radical scavengers such as superoxide dismutase. Studies also need to examine whether the changes we have noted here are different after longer term hypertension. Finally, it

would be useful to determine whether the coronary vascular dysfunction we have observed can be reversed after anti-hypertensive treatment.

APPENDIX

ABBREVIATIONS USED

APPENDIX

ABBREVIATIONS USED

$+dp/dt_{max}$	Maximum rate of left ventricular pressure development
ACh	Acetylcholine
ADP	Adenosine diphosphate
ANP	Atrial natriuretic peptide
AOP	Mean aortic pressure
AT	Atenolol
AT_1	Angiotensin II receptor – subtype 1
ATP	Adenosine triphosphate
BDK	Bradykinin
BNP	Brain natriuretic peptide
Ca^{2+}	Calcium ion
cAMP	Cyclic adenosine monophosphate
CBP	Mean coronary blood pressure
CFV	Coronary flow velocity
cGMP	Cyclic guanosine monophosphate
CGRP	Calcitonin-gene-related peptide
CNS	Central nervous system
CO	Cardiac output
DAG	Diacylglycerol
EDHF	Endothelial-derived hyperpolarizing factor
EDRF	Endothelial-derived relaxing factor
eNOS	Endothelial – nitric oxide synthase
ET	Endothelin
FAD	Flavin adenine dinucleotide
FMN	Flavin adenine mononucleotide
H^+	Hydrogen ion
HR	Heart rate
ICI	ICI-118,551 (selective β_2-blocker)
IP_3	Inositol 1,4,5-triphosphate
ISO	Isoproterenol
K^+	Potassium ion
LNA	Nω-nitro-L-arginine
LVP	Left ventricular pressure
LVSP	Left ventricular systolic pressure
M receptors	Muscarinic cholinergic receptors
NADPH	Nicotinamide adenine dinucleotide phosphate

NE	Norepinephrine
NO	Nitric oxide
NPY	Neuropeptide Y
PGI_2	Prostacyclin
RVH	Renovascular hypertension
SHR	Spontaneously hypertensive rats
SNP	Sodium nitroprusside
TRB	Terbutaline