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COMMENTARY

CORONARY ARTERY SPASM

MULTIPLE CAUSES AND MULTIPLE ROLES IN HEART DISEASE

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This essay deals with a body of recent clinical and experimental findings that reinforce the concept that adverse coronary smooth muscle activity, also called spasm, is not a peculiar occurrence limited to an arcane form of angina, but a common pathogenic element in acute myocardial infarction and in sudden cardiac death, the leading causes of death in our society. I will consider here the likelihood that the endothelium has only a limited secondary role in spasm, and will explore the categories of vasoactive substances that might be implicated directly in acute coronary contraction. The main theme of this article, however, is to foster the view that spasm is multifactorial; that intrinsic smooth muscle cell mechanisms are implicated intimately in spasm, acting sometimes alone and sometimes in concert with vasoconstrictors. Approaches to solving what has now emerged as a central problem in vascular biology will be explored.

CONTRACTILE DYSFUNCTION

Although contractile dysfunction is acknowledged to be an integral part of several major cardiovascular diseases, in particular hypertension and congestive heart failure, its contribution to coronary artery disease generally has been depreciated. Myocardial infarction and sudden cardiac death involve primarily large coronary arteries, but these conditions are most often attributed to the hemodynamic consequences of intraluminal plaque growth and vessel narrowing, culminating in thrombotic occlusion, rather than to the contractile characteristics of coronary vascular smooth muscle [1, 2].

The susceptibility of atherosclerotically narrowed vessel segments for thrombosis is assumed to be linked to their altered geometry and flow characteristics, against the background of endothelial damage, common at such sites. Coronary thrombosis has been linked especially to breaks or ulcerations in the surface of atheromas, exposing underlying thrombogenic surfaces to the vascular lumen. The vessel walls, in such cases, are presumed to be so compromised by rigid components of atherosclerosis that significant smooth muscle contraction is out of the question [3]. This view is seemingly supported by several key observations. In particular, thrombus formation occurs in atherosclerotically narrowed but not in physically unobstructed segments of human coronaries perfused postmortem with blood [4], and also in animal coronaries at sites of mechanically induced stenoses [5, 6]; occlusive thrombi are found angioscopically in the majority of patients with acute infarction [7]; and thrombolytic therapy but not vasodilator therapy re-establishes flow during the initial stages of an infarct, while the residual coronary lumen at the infarct site remains structurally narrowed [2]. Surprisingly, these salient observations are not incompatible with a principal role for spasm, and it is submitted here that smooth muscle contraction acts both as initiator and as exacerbater of acute coronary events.

RECONSIDERATIONS

Lumen narrowing

Much new evidence questions the commonly held association of extensive atherosclerotic narrowing with sites of thrombus formation. It is now clear that angiographic scoring of plaque severity, location and morphology in screened populations of patients does not enable prediction of culpable sites in a subsequent thrombotic myocardial infarction [8–10]. Further, repeat angiograms reveal that most coronary obstructions show no significant progression in severity even over a 3-year observation period [11, 12]. Even when angiography is done shortly before an acute episode, the location of the future infarct cannot be predicted [13].

Individuals who do experience infarctions often have an angiographic history of relatively minor or negligible lesions, in contrast to those who live indefinitely with chronic ischemia but without infarction. This suggests that severe obstructions present less risk for infarction or sudden death, linked to those particular sites, than do minor obstructions. This lack of positive correlation between the severity of the lesion in the future

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infarct vessel and the development of a myocardial infarction indicates that progression is discontinuous or episodic. It suggests that structurally rigidified vessels are not responsible for most infarction, probably since slow progression of such lesions allows for collateral vessel growth. Instead, an abrupt change in coronary artery geometry likely occurs, such as might be provided by inappropriate and intense smooth muscle contraction.

Thrombolysis

Information obtained from thrombolytic therapy supports the view that an acute alteration in vessel dynamics occurs around the time of infarct. It appears that the high-grade residual stenosis persisting after thrombolytic therapy does not define the severity of the pre-existing atheroma, but reflects the accumulated thrombic elements overlying the atheroma, making the lesion and vessel narrowing appear more severe than it was at the onset of the infarct [10, 14, 15]. Although occlusions are found in nearly 90% of vessels studied angiographically within the initial few hours of infarction, infusion of thrombolytic agents yields progressive increases in lumen diameter [16–18]. Angiographic separation of the coronary atheroma from the thrombus in infarct patients reveals that the underlying atherosclerotic lesion generally is not even sufficient to impair materially blood flow [17].

Plaque morphology

Plaque morphology encourages the idea that smooth muscle spasm may be the acute event that precipitates thrombosis and infarction. First, the large majority of infarct-linked plaques are associated with coronary segments that clearly retain the capacity to contract. Although concentric plaques are relatively rigid, due to the deficit in smooth muscle and the splinting effect of extracellular components, they account for less than a third of the total plaque number [19]. The much more common lesion, and the type linked to myocardial infarction and sudden cardiac death, is the eccentric lesion, which retains a disease-free wall arc length that represents a significant portion of the total vessel circumference, providing a considerable amount of smooth muscle, even when lumen obstruction is more than 50% [20].

Second, the eccentric lesion is prone to fracture. It often includes a space in the intima containing a pool of semi-liquid extracellular lipid held apart from the lumen by a cap of fibrous tissue that may be insufficiently supported by strands of collagen [19]. Intact eccentric lesions appear angiographically smooth, with tapered, intact surfaces, and those that have ruptured appear complex or rough surfaced, sometimes with narrow necks and luminal protrusions. A variety of work associates complex lesions with the major clinical ischemic conditions, in particular occlusive thrombus, infarction and sudden death [19, 21–27], and there is compelling reason to associate this pivotal sequence with vascular smooth muscle activity.

MECHANISMS OF PLAQUE RUPTURE

Plaque contents frequently are found dispersed deeply in the thrombus, indicating that plaque rupture likely precedes thrombus formation [28]. Soft lipid pools cannot bear significant stresses, and high tensile strength is concentrated at the ends of such plaque caps [29, 30]. These plaques are highly vulnerable to rupture and consequent hemorrhage into the plaque or, alternatively, emptying into the vessel lumen of atheromatous material, including lipid and collagen, with ensuing thrombosis and occlusion [14, 28]. In the view of some, plaque disruption may yield a large fissure through the fibrous cap of lipid-rich plaque, exposing collagen and fat to which platelet thrombi adhere [7, 13, 15].

Plaque rupture is deemed by most clinicians to be a random event, assignable to normal hemodynamic stresses [28], particularly when the fibrous cap is weakened by foam cell infiltration [31]. Sudden surges in blood pressure, repetitive dynamic stresses of pulsatile pressure causing plaque fatigue, or even repetitive bending during the cardiac cycle are thought by some to explain plaque rupture at weakened sites. However, coronary vascular tissue has a marked contractile potential that cannot be dismissed peremptorily when seeking the instigator of plaque rupture, and several clinical observations point to its decisive involvement.

SMOOTH MUSCLE AND RUPTURE

Autopsy studies indicated to Constantinides [21] that "a sudden explosion of pressure within the atherosclerotic arteries cracked their rigid walls," yielding thromboses and wall hemorrhages. Similarly, Hellstrom [32] postulated that "compressive forces of spasm could rupture the intima, and with soft plaques, extrude atheromatous material into the lumen." In a search for a morphological marker that would allow the postmortem diagnosis of coronary artery spasm, Factor and Cho [33] described medial smooth muscle contraction bands in some coronary arteries, correlated with atherosclerotic plaque ruptures and mural plaque hemorrhages, that appeared similar to those shown by others after experimental exposure of animals to high doses of vasoconstrictors [34].

The progression of coronary artery spasm to fatal infarct was documented angiographically, with autopsy demonstrating fracture of intimal collagen fibers and plaque rupture [35]. The direction of distortion of the torn intimal fibers of some coronary vessels, seen at autopsy, indicated to Lin *et al.* [23] "that the vector of disruptive forces proceeded from within the wall toward the lumen." They concluded that coronary artery spasm "can cause rupture of a soft, or semiliquid, atheromatous plaque in a way similar to volcanic eruption, with cholesterol crystals oriented to the rheological vector direction," and that the ruptured plaque appears to have been "continuously squeezed by spasm" with an irregular folding appearance of the fibrin thrombus. Such clinical observations, suggestive of intense and unremitting contraction, must be interpreted in terms of our knowledge of the contractile potential of the large epicardial arteries of the heart.

CONTRACTILE STATUS OF CORONARY ARTERIES

Human epicardial coronary artery segments show spontaneous tone and rhythmic motor activity *in vitro* [36, 37], as do those from most common laboratory animals, including dog, sheep, pig and cattle [36, 38]. Rhythmic contractions can reach amplitudes approximating 50% of maximal contractile capacity, a magnitude sufficient to alter blood flow. Our insight into *in vivo* coronary motor activity is limited, probably because of the vasodilator effects of the injected radiopaque chemicals, and to some extent the resolution characteristics of the cinematic equipment. However, epicardial arteries apparently maintain a basal level of contractile tone, because they are dilated by nitrite compounds, even in patients with diagnosed coronary artery disease [39–41].

Areas of coronary artery stenoses also show a basal level of contraction [39, 42, 43], with the large majority of eccentric stenoses, ranging in severity from 45 to 94% lumen obstruction, dilating an average of 49% to the combination of nitroglycerin and nifedipine [42], confirming smooth muscle activity in diseased segments.

An obstacle to the appreciation of spasm as a major player in infarction has been the notion that the large surface arteries of the heart are incapable of occlusive contractions, even when the tissues are provoked pharmacologically. It is now established that spastic occlusion of non-atherosclerotic arteries occurs spontaneously, or with pharmacological provocation, in patients with rest angina [44]. Further, a lumen occluding contraction is not necessary if the injurious sequence incorporates plaque rupture and thrombosis.

In my experience, the large epicardial coronary arteries *in vitro*, either perfused or mounted in muscle chambers, present a highly variable range of contractile performance to agonists, but contractility is much enhanced by the processes of priming and supersensitivity discussed later in this article. Full expression of the latent contractile potential of these vessels, in a region of plaque instability and thrombotic potential, might have devastating cardiovascular consequences.

DAMAGED ENDOTHELIUM AND SPASM

The endothelial monolayer synthesizes several potent vasodilators and vasoconstrictors, inhibits the aggregation of platelets and other cells, and according to some, hinders the penetration of exogenous vasoactive agents, including platelet products. However, the available evidence, summarized below, indicates that endothelial dysfunction is not the principal initiator of acute ischemic syndromes.

Access barrier function

The endothelium does not appear to protect the underlying arterial smooth muscle from agonists. Studies in which perfused segments of rabbit ear artery were cannulated at both ends, mounted in perfusion chambers, and selectively exposed to agonist through either the adventitial or intimal surface revealed that norepinephrine, potassium and methoxamine are just as potent in producing medial contractile responses when administered by the intraluminal (endothelial) route as extraluminally [45]. From another vantage point, Verbeuren *et al.* [46] perfused isolated dog coronary artery segments intraluminally with tritiated serotonin and found that segments without endothelium accumulated approximately twice the label of intact segments. Even if this difference in amine uptake and retention reflected totally the fate of free and active amine in the vicinity of the pertinent receptors, which is unlikely, it would translate at best into a modest impeding effect of the endothelium on the magnitude of contractions to serotonin, which are achieved over a 100-fold agonist concentration range.

Acute denudation should yield materially increased contractions if the endothelium is a barrier to the intimal penetration of agonist. My laboratory mounted cattle coronary artery segments as pairs in a sandwich configuration in muscle baths to compel access through the endothelial surface. These preparations contracted to serotonin, potassium chloride, and to a thromboxane A_2 analogue with a rate and response magnitude that were similar with the endothelium intact or denuded.* Also, preparations of cattle coronary artery segments in which the adventitial surfaces were coated with a barrier substance, such as saran wrap [47], to ensure an intimal route of entry, did not reveal an inhibitory effect of intact endothelium on agonist penetration, as measured by responses to several potent agonists.*

In vivo studies confirm that denudation of the endothelium does not directly alter responses to agonists. In one such study, done in anesthetized dogs, focal coronary artery denudation using a balloon-tipped catheter left unchanged the constrictor responses to intracoronary angiotensin and to phenylephrine [48]. Similarly, the effects of the thromboxane A_2 analogue, U 46619, on the diameter of the left anterior descending coronary in dogs did not differ when compared immediately before and after endothelial denudation [49].

Basal EDRF release

The endothelium is the source of EDRF† (NO), and a number of investigators consider the loss of endothelium-mediated relaxation to be the underlying common denominator in abnormal coronary constriction [50–52]. There is little doubt that release of EDRF provoked by acetylcholine and bradykinin can produce potent, albeit transient, relaxations of precontracted coronary epicardial arteries *in vitro*. However, such incited release tells little about the actual participation of endothelially liberated dilators in cardiovascular events.

Studies *in vitro*, utilizing mechanical denudation, show that endothelially produced vasodilators are not suppressing substantial inherent tone in cattle, pig and dog coronary arteries [53–55]. Pharmacological denudation of the endothelium has yielded mixed data, partially because of drug specificity problems.

* Kalsner S, unpublished data.

† Abbreviations: EDRF, endothelium-derived relaxing factor; L-NMMA, L-N^G-monomethyl arginine; and L-NOARG, L-N^G-nitro arginine.

Methylene blue, one of the key compounds used to define basal EDRF release, acts on the soluble guanylate cyclase enzyme located in the smooth muscle cells, making interpretation of tone changes difficult [56]. Additionally, methylene blue is non-specific, interfering with a number of cellular processes [57, 58]. My own experience with the compound is that it sometimes produces major contractions of coronary artery segments that cannot be duplicated by other inhibitors or by mechanical denudation, and therefore increases in basal tone with this compound do not certify the loss of a potent relaxation vector. In moderate concentrations, neither methylene blue nor the inhibitor hemoglobin contract human coronary arteries *in vitro* [59].

Arginine analogs are relatively specific inhibitors of nitric oxide synthesis, and they might provide more reliable information on the basal release of EDRF and its inhibitory impact on tone. Rabbit aorta rings show very slight contractions to L-NMMA (up to 300 μM) [60] and, according to others, neither L-NOARG (15 μM) nor L-NMMA (30 μM) contract aortic rings in the absence of agonists [61]. Similarly, the basal tone of human mammary artery rings *in vitro* were only very slightly increased in tension by L-NMMA (10^{-4}M) [62]. My own studies with cattle coronary rings do not show contractions to L-NMMA.*

Suppression *in vivo* of basally produced EDRF, either pharmacologically or by endothelial denudation, does not in itself free any significant amount of coronary muscle tone, and certainly does not trigger spasm. Balloon denudation of the left descending coronary artery in intact anesthetized dogs did not alter significantly the dimensions of the artery over a 120-min observation period [63], and similar findings have been made by others [48, 49]. Although the intracoronary administration of the nitric oxide synthesis inhibitor L-NMMA to patients blocks the vasodilator response to acetylcholine, confirming efficacy, it does not alter significantly the tone of the proximal left anterior descending artery and only very slightly, by 5.9%, that of the distal portion [64]. These findings are not surprising since human coronary arteries *in vivo* manifest basal tone, which is not in keeping with an endothelial override of coronary contractility.

Other work indicates that denudation of the endothelium does not alter significantly either the ED_{50} or the maximum contractile response to phenylephrine, histamine or serotonin in rabbit aorta [65]. Similarly, denudation of dog, pig and cattle coronary artery rings *in vitro* does not increase significantly contractions to phenylephrine, potassium chloride or prostaglandin $F_{2\alpha}$* [54, 66].

Studies *in vivo* support a minimal role of endothelial dilators in impeding contractility. For example, in intact anesthetized dogs the constrictor responses of the left descending coronary artery to phenylephrine and to angiotensin are similar in the presence and in the absence of endothelium [48]. It is possible, however, that the provoked release of vasodilators from the endothelium by certain exogenous vasoconstrictors, such as acetylcholine and serotonin, temper or counter their medial actions, and account for potentiated responses observed in some denuded tissues in certain species [48, 51, 67]. But there is substantial disagreement as to whether endothelially mediated dilator responses to serotonin and norepinephrine operate effectively in human coronaries [39, 59, 68].

Endothelium and atherosclerosis

How do we explain those experiments that seem to link damaged endothelium with increased contractions to agonists? A number of them compound denudation with a battery of additional acute and chronic treatments, and in humans, with the longstanding conditions of age, hypercholesterolemia and atherosclerosis. Several seemingly incontrovertible observations strongly negate the notion that structural or functional impairment of endothelium, alone, has catastrophically negative effects on coronary diameter. Depressed release of EDRF by acetylcholine, shear stress, or exercise occurs too frequently in ageing or hypercholesterolemic individuals [50, 69–71] to explain the triggering spasm of angina, or the plaque rupture of infarction and sudden death. Some aspects of endothelium-mediated dilation are lost or attenuated early in the course of atherosclerosis, when vessels have minor surface irregularities as assessed angiographically, and even in smooth surfaced vessel segments in otherwise atherosclerotic beds [52, 69, 71, 72]. In fact, compromised endothelium-mediated relaxation may be common in the general population at any age [73], including smokers [74], and also has been noted in an animal model of hypertension [75]. In one relevant study, examining the responses of human vessel segments removed during surgery, only half of them showed endothelial-mediated relaxations to acetylcholine or to the ionophore A23187, and some normal arteries from young subjects failed to show endothelial responses [59].

Prostacyclin relaxes large coronary arteries, and its release is activated by shear stress and hypoxia, but the above-quoted experiments on mechanical denudation, and other work [76], suggest it contributes little to endothelium-dependent relaxation. Additionally, the endothelium releases a hyperpolarizing factor that cannot be explained by the action of EDRF [77], but again, based on the denudation experiments described above, its contribution to spasm does not appear substantial. Other work has shown that vasodilator prostaglandins, distinct from prostacyclin, perhaps deriving from the smooth muscle itself, may contribute to coronary tone in cattle and perhaps other species [78].

PHARMACOLOGICAL CONSIDERATIONS

A number of substances potentially are spasmogenic, particularly when working against a background of heightened coronary reactivity. The initiation of spasm could involve one vasoconstrictor, or a combination of them. In terms of vascular architecture, candidates range from adrenergic and cholinergic neurotransmitters and their associated cotransmitters, derived from the nerve plexuses

* Kalsner S, unpublished data.

embedded in the adventitial-medial border [53, 79–81], to the peptide endothelin released from the inner endothelial lining [82, 83]. Endothelial and perhaps muscle cells additionally synthesize several prostaglandins, thromboxanes and lipoxygenase products of arachidonic acid with contractile properties [84], and their dysfunctional release cannot be ruled out, but is presently uncertain [75, 85–87]. Murally invading inflammatory cells may also contribute vasoactive compounds [88], but we know little about them.

The bulk of studies on adrenergic control have examined regulation of arterioles and precapillary vessels and have not focused on the large epicardial arteries of the heart, which appear minimally controlled by adrenergic mechanisms [89, 90]. Human coronary artery strips *in vitro* contract slightly to low-frequency stimulation, reflecting contributions from both adrenergic and cholinergic nerves, as responses are partially antagonized by phentolamine and atropine [53]. Periarterial stimulation of a perfused cattle myocardial slab *in vitro* elicits a potent vasoconstrictor response that is antagonized by atropine and potentiated by physostigmine [91], but this may also involve smaller downstream vessels. Against a background of enhanced vascular reactivity, neurogenic activity may provide sufficient contractile input to rupture plaque.

Serotonin and histamine are prime candidates in the elusive search for mediators of spasm. They can potently contract the largest epicardial arteries, particularly when reactivity is increased. Kalsner and Richards [92] found that human coronary artery segments contain very substantial stores of histamine and serotonin, and that the amount of histamine is elevated very significantly in the arteries of older patients and in those whose deaths are attributed to coronary artery disease. These autocoids might be liberated by provoking stimuli, such as in an antigen–antibody reaction.

PLATELETS: THE RELEASE OF VASOACTIVE AGENTS AND REINFORCEMENT OF SPASM

The second phase of smooth muscle involvement is defined here as that which follows the spasm that initiates plaque rupture or severe platelet aggregation. It incorporates vasoactive products of platelet aggregation and release, such as thromboxane and serotonin, as well as those of murally invading cell types [88]. It should be noted, however, that areas of damaged endothelium and platelet deposition are common in the ageing human coronaries, as discussed above, and platelet deposition alone does not initiate an occlusive thrombus [19].

Animal models used to simulate acute coronary artery disease usually couple inflicted endothelial damage with mechanical compression of coronary artery tissue and intrusive promotion of thrombus [93–95]. Since the pathogenetic elements in human acute ischemic syndromes are not yet established, however, no animal model can be presumed to replicate the human condition. This is particularly evident when it is considered that most human disease involves arteries of 45- to 80-year-old patients, for whom there is no matching animal counterpart.

In a favored model initially described by Folts *et al.* [5], a rigid plastic cylinder is placed around the outside of an epicardial artery of dogs, sufficient to compress the vessel and reduce the lumen by about 60%. The intima is wrinkled and distorted, the endothelium damaged and the subendothelium exposed [96]. Periodic decreases in flow, occurring several times an hour and lasting minutes, begin promptly after creating the stenosis. The cyclic flow irregularities have been attributed, in a series of publications, to platelet thrombi forming and dislodging periodically in the narrowed lumen [6]. Cyclic flow deteriorates to persistently low flow usually within hours due to an occlusive thrombus, confirmed at the constrictor site [6, 97]. One essential thrust of this research has been to substantiate the view that platelet-mediated obstruction of stenosed and endothelially injured vessels is the critical event in the abrupt progression from a chronic stable myocardial picture to an unstable one [6]. However, mechanical compression of an artery to simulate atherosclerotic narrowing must also be considered a simulation of persistent coronary artery spasm.

Cyclic flow variations are antagonized by inhibitors of cyclooxygenase and thromboxane A_2 synthetase, and by the serotonin S_2 receptor antagonist ketanserin. These effects have been attributed, with few exceptions [97], to inhibition of platelet aggregation and release rather than to block of vasoconstriction [6, 93, 98]. But thromboxane A_2 is synthesized and released in large quantities by aggregating platelets and is a vasoconstrictor as well as a platelet-aggregating agent, despite its brief half-life of approximately 30 sec [99]. Thromboxane analogs, such as U44069 and U44619, potently contract dog, monkey, human and cattle coronaries [100–102]. Similarly, serotonin is released in very high peak concentration during platelet aggregation [51].

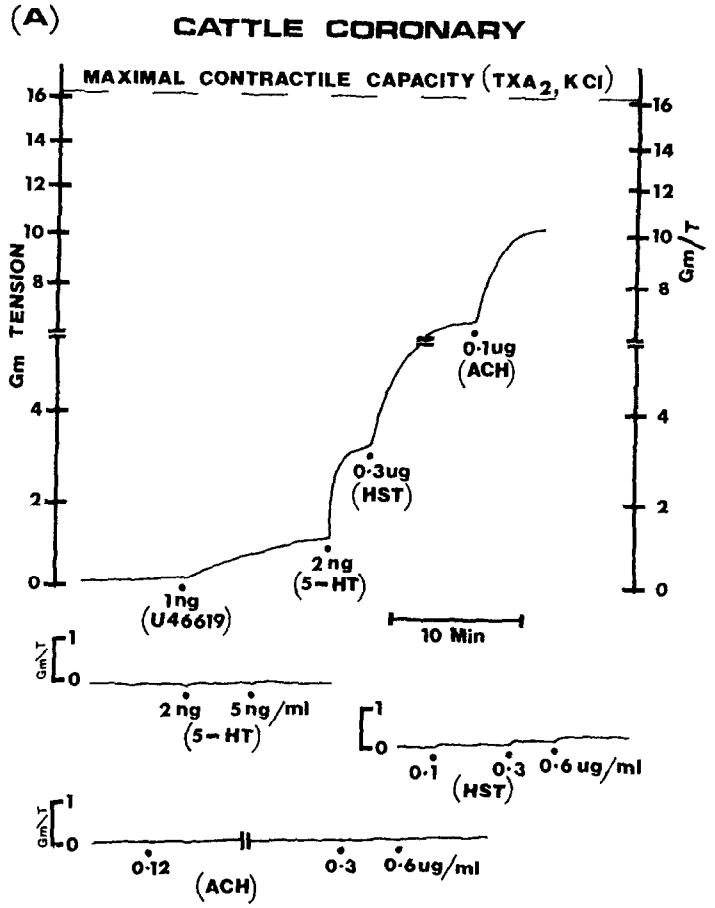
My own work with isolated cattle hearts, perfused with Krebs–Henseleit solution rather than blood, seems to confirm a role for motor activity both in the initial and in the secondary phases following mechanical occlusion in the absence of blood-borne vasoconstrictors. We placed a plastic constrictor around a doubly cannulated and isolated branch of the left coronary artery in an *in vitro* cattle heart preparation and monitored flow under constant perfusion pressure. Immediately following placement of the occluder, flow was substantially reduced, and then returned partially to control values, coupled with cyclic flow oscillations that were antagonized by vasodilators.* Undoubtedly, *in vivo*, platelet released vasoconstrictors contribute additionally, and potently, to flow oscillations.

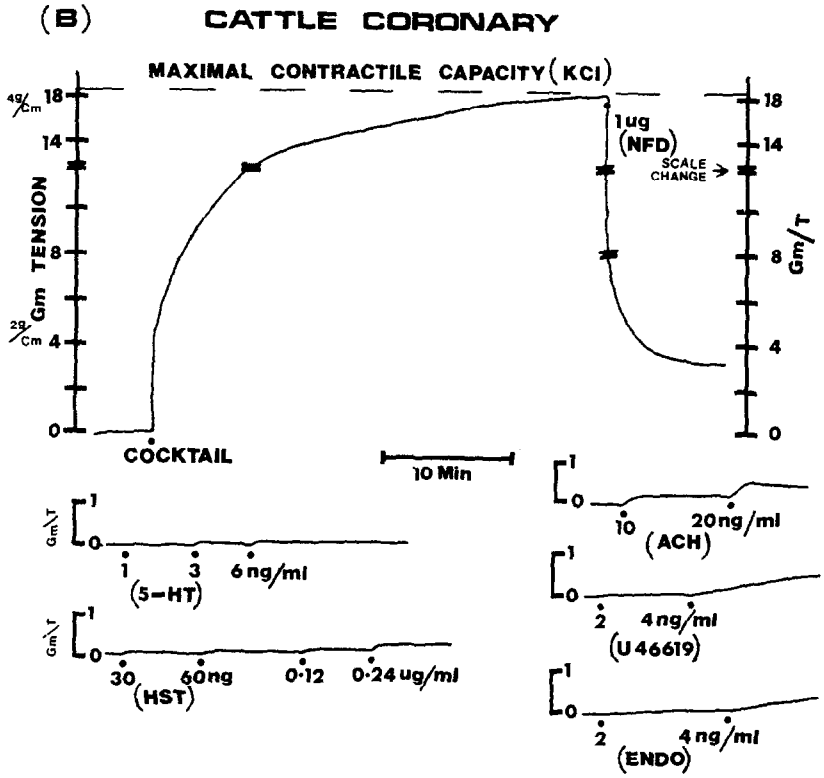
THREE ALTERNATE SCENARIOS

Hyperreactivity

A possible paradigm for the spasm that initiates infarction and sudden cardiac death invokes smooth muscle cell supersensitivity to contracting agents,

* Kalsner S, unpublished data.


(A)
CATTLE CORONARY
MAXIMAL CONTRACTILE CAPACITY (TXA2, KCl)
Gm TENSION
Gm/T
0·1ug (ACH)
0·3ug (HST)
2 ng (5-HT)
1ng (U46619)
10 Min
2 ng 5 ng/ml (5-HT)
0·1 0·3 0·6 ug/ml (HST)
0·12 0·3 0·6ug/ml (ACH)


(B)
CATTLE CORONARY
MAXIMAL CONTRACTILE CAPACITY (KCl)
Gm TENSION
Gm/T
1ug (NFD)
SCALE CHANGE
COCKTAIL
10 Min
1 3 6 ng/ml (5-HT)
30 60ng 0·12 0·24 ug/ml (HST)
10 20ng/ml (ACH)
2 4ng/ml (U46619)
2 4 ng/ml (ENDO)

with lowered thresholds (affinity) and increased maximal responses (efficacy) [92, 103]. It is well established that coronary artery segments are hyperreactive to diverse stimuli in rest angina [44], and this is not limited to atherosclerotic vessels. The coronary arteries of some patients in a pre-infarction and post-infarction state also seem hyperreactive to pharmacological provocation, reacting with focal spasm [104, 105].

My own laboratory found that the contractility to serotonin, histamine and norepinephrine of epicardial coronary arteries removed soon after death from hearts of cardiac patients was much increased over the entire concentration–response curve, compared to non-cardiac tissues [92]. Maximal responses to histamine were over 1000-fold greater in the cardiac group, and those to serotonin were increased over 400%. The enhanced efficacy provides a background against which any available vasoactive agent might cause spasm. Similarly, Allen *et al.* [106] reported that non-atherosclerotic human coronary segments *in vitro* responded with weak contractions to leukotrienes but responses of atherosclerotic vessels were much increased. Further, studies in miniature pigs and monkeys chronically fed a high cholesterol diet, in some cases after inflicted endothelial damage, support the concept of enhanced vascular reactivity in human coronary disease [65, 107–111].

Priming

Priming is a term that I use to describe a phenomenon examined in some detail in my laboratory, and it may explain the reinforcing spasm of unstable ischemic conditions that involve liberated platelet products. Priming means that when subthreshold or threshold concentrations of agonists that act through discrete receptors are present simultaneously, any single further agonist addition, even in threshold concentration, elicits substantial, even near maximal contractions or spasm. This process is a form of synergism. In primed coronary artery segments, virtually any compound with minimal vasoconstrictor activity, even in minimal concentration, may become a potent spasmogen. These substances may be added to the external phase sequentially or together as a cocktail (Fig. 1, A and B). This phenomenon likely occurs *in vivo* and negates the requirement for the culpable agonist to have sufficient potency and concentration, on its own, to induce spasm.

Spasm and no spasmogens

Propagated responses. Propagated contractions occur in large coronary arteries and may invoke spasm. For example, the frequently reported induction of spasm by an intruding coronary catheter [112] indicates excitation spread some finite distance through an arterial segment. This process illustrates spasm without a spasmogen. The vasospasm of rest angina occurs repeatedly in patient-specific coronary artery loci, pointing again to an excitatory alteration in vascular cells [101, 113].

Spontaneously generated contractions probably involve membrane electrical activity, common to a variety of smooth muscles, particularly the intestinal tract [114]. With respect to blood vessels, electrically induced tone changes not involving neurotransmitters have been associated with the single unit type of vascular smooth muscle, such as arterioles and some veins. It has been customary to regard coronary arteries as multiunit smooth muscle, each cell discrete from its neighbors, controlled by neurotransmitters and hormones, rather than as syncytial smooth muscle capable of generating and coordinating electrical activity between cells, but even the largest epicardial arteries demonstrate substantial rhythmic activity [36, 37, 115].

It is known that a single pacemaker cell can drive the contractility of a relatively large area of human and pig coronary tissue [116]. Such responses are likely mediated by intercellular pathways of low electrical resistance, namely connexons or gap junctions, similar in protein composition to those in cardiac tissue [117]. The conducted electrical activity must activate voltage-sensitive channels and transport processes in each responsive coronary artery cell. For example, spontaneous calcium transients, or oscillations in cytoplasmic free calcium, have been recorded in multilayered cultures of rat aortic smooth muscle cells and seem to represent synchronous activity in electrically coupled cells [118]. Such processes, apart from any inciting agonist–receptor combination, may be fundamental to understanding spasm.

Electrically induced responses. My laboratory has developed a means of activating relaxation and contraction processes that seem to mimic components of spontaneous motor activity. A voltage-sensitive relaxation cascade can be triggered off in contracted coronary arteries of animals and humans by a few very brief and innocuous externally applied electrical pulses, far below the number needed to activate neurogenic mechanisms in arterial tissue [119]. Even a single pulse of 0.1-msec duration, delivered through transmurally placed electrodes to a superfused coronary artery ring or strip, in a state of spontaneous or stimulant-induced tone, profoundly blocks that

Fig. 1. Effects of vasoconstrictors alone and in threshold or subthreshold combination on coronary artery contractions. (A) Responses to serotonin (5-HT), histamine (HST), acetylcholine (ACH) and U46619 individually and in sequential combination on isolated cattle epicardial preparations. Concentrations of each compound are as shown. (B) Same as (A) except that endothelin (ENDO) was also used, and all drugs were added either individually or together as a cocktail at the highest indicated concentrations of each. The calcium channel antagonist nifedipine (NFD) was added as indicated. All preparations in (A) and (B) are from the same coronary vessel and heart. The vertical axes indicate grams of tension in isometric recording (note the scale changes on the ordinate) and the horizontal axis indicates time. Dashed lines indicate the maximal contractile tension that can be pharmacologically produced in these vessels.

tone. Relaxation does not begin promptly with stimulation but only after a delay of about 20 sec, likely signifying the involvement of intermediary metabolic processes, and minutes are required for the full effect and the return of agonist-induced tone [119]. Repeated stimulation, even with a single 0.5-msec pulse, once every minute, usually ensures sustained blockade of pre-existing contractions to 5-hydroxytryptamine, the thromboxane A_2 analogue (U46619) and endothelin.

Anti-oxidants to inhibit free radicals, including superoxide dismutase and catalase, do not reduce the relaxations and neither does endothelial denudation, tetrodotoxin, or antagonists of prostaglandin synthesis. Utilizing cascade style experiments, it was determined that no vaso-relaxant substance was released by the stimulated tissue [101, 119].

Mechanisms of relaxation and contraction. Studies on the ionic dependency of the observed relaxations, and experiments with pharmacological antagonists of membrane transport systems, indicated that the applied stimulation pulses activated voltage-sensitive calcium-dependent potassium channels, and to some extent Na^+, K^+-ATPase, presumably by modifying protein conformation with a resultant hyperpolarization* [101, 119]. Hyperpolarization or repolarization, in turn, likely activates calcium sequestration processes, namely calcium-ATPases that sequester or extrude intracellular calcium, and also possibly the forward mode of the Na^+–Ca^{2+} exchanger [120], allowing the dephosphorylation of myosin light chain and vascular relaxation to proceed.

Whereas vessels in a state of contraction relaxed to stimulation, we soon found that when tone was minimal they contracted to brief electrical pulses, even in the presence of adrenergic and cholinergic antagonists and tetrodotoxin.

Stimulation with one pulse of 0.5-msec duration every 3 min, or even a single 0.5-msec pulse, led to large contractions, often sustained over many minutes. These contractions were not reduced by anti-oxidants and were equivalent to those produced in responsive vessels by potent coronary stimulants such as endothelin, and the thromboxane A_2 analog U46619, given in concentrations in excess of the EC_{50} values. We found, surprisingly, that these contractions seem mediated largely by the release of calcium from voltage-sensitive cellular stores, as they are largely resistant to calcium channel antagonists and zero calcium Krebs solution [121].

Contractions to 1 or 5, 0.5-msec pulses were sometimes of very large amplitude and, in some preparations stressed by prolonged storage, they did not reverse even over an hour-long observation period (Fig. 2). *In vivo*, coronary artery smooth muscle cells are damaged slowly over time by atherosclerosis and medial necrosis, and these might be counterparts to our storage *in vitro*.

IMPLICATIONS OF SPONTANEOUS ACTIVITY

Clinical investigators have not integrated spontaneous motor activity and defects in it into the mix of factors at the root of coronary artery disease. Can the initiating and plaque rupturing spasm of infarction ensue from a dysfunctional smooth muscle cell physiology in the face of an intrinsically generated and conducted electrical signal? If so, the key to vasospasm in human coronary artery disease would lie not in the quantity of impinging spasmogen, but in the quality of the conducted signal and in those processes that normally control the contractile machinery through the accumulation and removal of second messengers at intracellular sites of action in individual smooth muscle cells.

What about the evidence implicating chemicals in the initiation and maintenance of spasm? It is possible that the mechanism(s) sensitized by agonists during priming may be the very processes activated by our stimulation pulses. My laboratory found that very low, even subthreshold concentrations of any one of several spasmogens, including thromboxane, endothelin and serotonin, or elevated potassium, markedly potentiate the magnitude of the contractions elicited by 1 or 5 stimulation pulses each of 0.5-msec duration [121].

Ergonovine incites spasm in patients with rest angina and in some with recent infarction, reflecting increased responsivity of relevant coronary segments, and this may tie in to the mechanism described here; we find that the responses to even a single stimulation pulse are greatly magnified in size and prolonged in duration by subthreshold concentrations of ergonovine.

AGEING AND CORONARY REACTIVITY

In distinct contrast to most ageing biological systems, older human coronary arteries, examined *in vitro*, show heightened spontaneous motor activity and increased reactivity to pharmacological stimuli *in vitro* [92]. This is also the case in vessel segments taken from the hearts of those whose deaths were attributed by the pathologist to cardiac disease, and whose hearts showed myocardial evidence of old or recent infarcts [92]. Many of these vessels showed substantial atherosclerosis and mineralization.

PRECISE MECHANISM OF SPASM

As to the precise subcellular mechanism(s) involved in spasm, much work needs to be done. Enhanced magnitude and prolonged duration of response to a contractile signal likely indicate a defect in the removal of calcium from its intracellular sites of action, or a failure to reverse the processes activated by calcium [91]. We know extremely little about the processes that hold or remove calcium at its site of action, or from its combination with calmodulin, or even if the continued presence of calcium is necessary for contraction. Until such processes are better understood, the precise defect in tone control will probably remain obscure. An increased sensitivity of contractile mechanisms independently of calcium also cannot be excluded. We need to explore the multifactorial processes governing tone, including Na^+, K^+-ATPase, the Na^+-Ca^{2+} exchanger, cyclic nucleotides, K^+-dependent calcium channels and calcium sequestration

* Kalsner S and Yao JA, unpublished data.

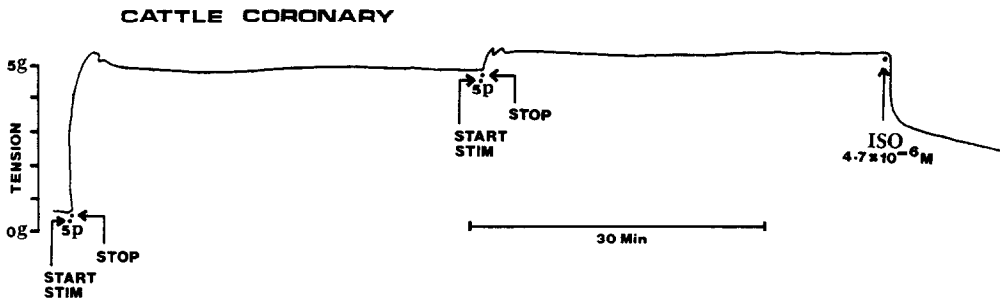


Fig. 2. Cattle coronary artery stimulated twice with 5 pulses of 0.5-msec duration at 0.5 Hz. Dots indicate onset and offset of stimulation. Persistent contractions to stimulation can be reversed by certain vasodilators, e.g. isoproterenol (ISO), as shown. The vertical scale indicates grams tension of isometric recording of superfused coronary artery preparations stimulated as described in text. The horizontal scale shows time.

processes. Is the actin-myosin system and dephosphorylation of the 20,000 Da myosin light chain by myosin light chain phosphatase itself impaired in spasm?

OTHER IMPLICATIONS

Interestingly, cholesterol and atherosclerosis may interact with intrinsically generated tone signals. Exogenous cholesterol is well known to incorporate itself into cell membranes and to alter vascular reactivity, presumably by modifying the physical state of membranes and altering ion channel conformation. Gleason *et al.* [122] found that norepinephrine-induced contractions and calcium uptake were increased in rabbit carotid artery segments perfused with cholesterol-enriched liposomes. Voltage-clamping studies reveal that single channel conductance is altered in synthetic membranes by cholesterol enrichment, and aortic plasma membrane transport of calcium is much increased in experimental atherosclerosis in rabbits [123]. Cholesterol depresses the activity of sarcoplasmic reticulum Ca^{2+}-ATPase in skeletal muscle, which removes free intracellular calcium [124]. Should cholesterol alter coronary artery membrane function similarly, any conducted electrical response or agonist-initiated response would be both prolonged and enhanced.

SUMMARY

Myocardial infarction and sudden cardiac death may be initiated by a sudden intense localized contraction of coronary artery smooth muscle. When this event occurs around a vulnerable eccentric lipid-filled plaque, rupture and extrusion of plaque contents and exposure of collagen occur. This may sometimes be a silent and self-limiting event; other times it leads to thrombus formation. A second wave of spasm due to accumulated platelet and inflammatory mediators may compound the contractile consequences of the initiating event. Spasm involves intrinsic smooth muscle cell electrical mechanisms, hyper-responsive cells, and multiple agonists that synergize their actions, and the involvement of each mechanism varies at different times in the sequence of vascular occlusion. Study of spasm requires vascular systems that adequately model coronary artery responses of the ageing human heart. As previously emphasized, tissues obtained postmortem, and when possible from recipients during heart transplants, must be integral to theory building, alongside animal models, despite the experimental limitations such tissues impose. A multidisciplinary approach, at all levels of vascular physiology and pharmacology, will be necessary to understand coronary motor activity and human heart disease.

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REFERENCES

1. Horn H and Finkelstein LE, Arteriosclerosis of the coronary arteries and the mechanism of their occlusion. *Am Heart J* **19**: 655–682, 1940.
2. Laffel GL and Braunwald E, Thrombolytic therapy: A new strategy for the treatment of acute myocardial infarction. *N Engl J Med* **311**: 710–717, 770–776, 1984.
3. Blumgart HL, Schlesinger MJ and Davis D, Studies on the relation of the clinical manifestations of angina pectoris, coronary thrombosis and myocardial infarction to the pathologic findings. With particular reference to the significance of the collateral circulation. *Am Heart J* **19**: 1–91, 1940.
4. Uchida Y, Masuo M, Tomaru T, Kato A and Surimoto T, Fiberoptic observation of thrombosis and thrombolysis in isolated human coronary arteries. *Am Heart J* **112**: 691–696, 1986.
5. Folts JD, Crowell EB Jr and Rowe GG, Platelet aggregation in partially obstructed vessels and its elimination with aspirin. *Circulation* **54**: 365–370, 1976.
6. Willerson JT, Eidt JF, McNatt J, Yao SK, Golino P, Anderson HV and Buja LM, Role of thromboxane and serotonin as mediators in the development of spontaneous alterations in coronary blood flow and neointimal proliferation in canine models with chronic

coronary artery stenoses and endothelial injury. *J Am Coll Cardiol* **17**: 101B–110B, 1991.
7. Mizuno K, Miyamoto A, Satomura K, Kurita A, Arai T, Sakurada M, Yanagida S and Nakamura H, Angioscopic coronary macromorphology in patients with acute coronary disorders. *Lancet* **337**: 809–812, 1991.
8. Bemis CE, Gorlin R, Kemp HG and Herman MV, Progression of coronary artery disease: A clinical arteriographic study. *Circulation* **47**: 455–464, 1973.
9. Bruschke A, Wijers T, Kolsters W and Landmann J, The anatomic evaluation of coronary artery disease demonstrated by coronary angiography in 256 non-operated patients. *Circulation* **63**: 527–540, 1981.
10. Little WC, Constantinescu M, Applegate RJ, Kutcher MA, Burrows MT, Kahl FR and Santamore WP, Can coronary angiography predict the site of a subsequent myocardial infarction in patients with mild-to-moderate coronary artery disease? *Circulation* **78**: 1157–1166, 1988.
11. Stone PH, Gibson CM, Pasternak RC, McManus K, Diaz L, Boucher T, Spears R, Sandor T, Rosner B and Sacks FM for the Harvard Atherosclerosis Reversibility Project Study Group, Natural history of coronary atherosclerosis using quantitative angiography in men, and implications for clinical trials of coronary regression. *Am J Cardiol* **71**: 766–772, 1993.
12. Singh RN, Progression of coronary atherosclerosis: Clues to pathogenesis from serial coronary arteriography. *Br Heart J* **52**: 451–461, 1984.
13. Moise A, Theroux P, Taeymans Y, Descoings B, Lesberance J, Waters DD, Pelletier GB and Bourassa MG, Unstable angina and progression of coronary atherosclerosis. *N Engl J Med* **309**: 685–689, 1983.
14. Woolf N and Davies MJ, Interrelationship between atherosclerosis and thrombosis. In: *Thrombosis in Cardiovascular Disorders* (Eds. Fuster V and Verstraete M), pp. 41–77. WB Saunders, Philadelphia, 1992.
15. Ambrose JA, Tannenbaum MA, Alexopoulos D, Hjemdahl-Monsen CE, Leavy J, Weiss M, Borrico S, Gorlin R and Fuster V, Angiographic progression of coronary artery disease and the development of myocardial infarction. *J Am Coll Cardiol* **12**: 56–62, 1988.
16. DeWood MA, Spores J, Nostske MD, Mouser LT, Burroughs R, Golden MS and Lang HT, Prevalence of total coronary occlusion during the early hours of transmural myocardial infarction. *N Engl J Med* **303**: 897–902, 1980.
17. Brown BG, Gallery CA, Badger RS, Kennedy JW, Mathey D, Bolson EL and Dodge HT, Incomplete lysis of thrombus in the moderate underlying atherosclerotic lesion during intracoronary infusion of streptokinase for acute myocardial infarction: Quantitative angiographic observations. *Circulation* **73**: 653–661, 1986.
18. Armstrong PW, Beck CE, Freeman MR, Chisholm RJ, Canepa-Anson R, Casella L and Kent RS, Prolonged tPA infusion in acute myocardial infarction: Impact on thrombolysis and recurrent ischemia. *Circulation* **76** (Suppl IV): IV–452, 1987.
19. Davies MJ, Macroscopic or microscopic view of coronary thrombi. *Circulation* **82**: 1138–1146, 1990.
20. Saner HE, The disease-free wall in coronary atherosclerosis. *J Am Coll Cardiol* **6**: 1096–1099, 1985.
21. Constantinides P, Plaque fissures in human coronary thrombosis. *J Atheroscler Res* **6**: 1–17, 1966.
22. Ridolfi RL and Hutchins GM, The relationship between coronary artery lesions and myocardial infarcts: Ulceration of atherosclerotic plaques precipitating coronary thrombosis. *Am Heart J* **93**: 468–486, 1977.
23. Lin C-S, Penha PD, Zak FG and Lin JC, Morphodynamic interpretation of acute coronary thrombosis, with special reference to volcano-like eruption of atheromatous plaque caused by coronary artery spasm. *Angiology* **39**: 535–547, 1988.
24. Taeymans Y, Théroux P, Lespérance J and Waters D, Quantitative angiographic morphology of the coronary artery lesions at risk of thrombotic occlusion. *Circulation* **85**: 78–85, 1992.
25. Fuster V, Badimon L, Badimon JJ and Chesebro JH, The pathogenesis of coronary artery disease and the acute coronary syndromes. (First of two parts). *N Engl J Med* **326**: 242–250, 1992.
26. Davies MJ, Anatomic features in victims of sudden coronary death. *Circulation* **85**: I19–I24, 1992.
27. Chapman I, Morphogenesis of occluding coronary artery thrombosis. *Arch Pathol* **80**: 256–261, 1965.
28. Falk E, Plaque rupture with severe pre-existing stenosis precipitating coronary thrombosis: Characteristics of coronary atherosclerotic plaques underlying fatal occlusive thrombi. *Br Heart J* **50**: 127–134, 1983.
29. Richardson PD, Davies MJ and Born GV, Influence of plaque configuration and stress distribution on fissuring of coronary atherosclerotic plaques [see comments]. *Lancet* **2**: 941–944, 1989.
30. Cheng GC, Loree HM, Kamm RD, Fishbein MC and Lee RT, Distribution of circumferential stress in ruptured and stable atherosclerotic lesions. A structural analysis with histopathological correlation. *Circulation* **87**: 1179–1187, 1993.
31. Lendon C, Davies M, Born G and Richardson P, Atherosclerotic plaque caps are locally weakened when macrophage density is increased. *Atherosclerosis* **65**: 302–310, 1991.
32. Hellstrom HR, Evidence in favor of the vasospastic cause of coronary artery thrombosis. *Am Heart J* **97**: 449–452, 1979.
33. Factor SM and Cho S, Smooth muscle contraction bands in the media of coronary arteries: A postmortem marker of antemortem coronary spasm. *J Am Coll Cardiol* **6**: 1329–1337, 1985.
34. Joris I and Majno G, Endothelial changes induced by arterial spasm. *Am J Pathol* **102**: 346–358, 1981.
35. Morimoto SI, Shiga Y, Hiramitsu S, Yamada K, Nomura S, Miyagi Y, Nomura M and Mizuno Y, Plaque rupture possibly induced by coronary spasm. *Jpn Circ J* **52**: 1286–1292, 1988.
36. Kalsner S, Cholinergic mechanisms in human coronary artery preparations: Implications of species differences. *J Physiol (Lond)* **358**: 509–526, 1985.
37. Kalsner S, Coronary artery reactivity in human vessels: Some questions and some answers. *Fedn Proc* **44**: 321–325, 1985.
38. Henry PD, Coronary artery spasm. In: *Physiology and Pathophysiology of the Heart* (Ed. Sperelakis N), pp. 819–833. Martinus Nijhoff, Boston, 1984.
39. McFadden EP, Clarke JG, Davies GJ, Kaski JC, Haider AW and Maseri A, Effect of intracoronary serotonin on coronary vessels in patients with stable angina and patients with variant angina. *N Engl J Med* **324**: 648–654, 1991.
40. Tousoulis D, Davies G, McFadden E, Clarke J, Kaski J and Maseri A, Coronary vasomotor effects of serotonin in patients with angina. *Circulation* **88**: 1518–1526, 1993.
41. Maseri A, Newman C and Davies G, Coronary vasomotor tone: A heterogeneous entity. *Eur Heart J* **10** (Suppl F): 2–5, 1989.
42. Rafflenbeul W and Lichtlen P, Quantitative coronary angiography—Evidence of a sustained increase in vascular smooth muscle tone in coronary artery stenosis. *Z Kardiol* **72**: 87–91, 1983.
43. Brown BG, Bolson EL and Dodge HT, Dynamic

mechanisms in human coronary stenosis. *Circulation* **70**: 917–922, 1984.
44. Maseri A, L'Abbate A, Pesola A, Ballestra AM, Marzilli M, Maltinti G, Severi S, De Nes DM, Parodi O and Biagini A, Coronary vasospasm in angina pectoris. *Lancet* **1**: 713–717, 1977.
45. Kalsner S, Effects of the inhibition of noradrenaline uptake and synthesis on the maintenance of the response to continuous nerve stimulation in the central artery of the rabbit ear. *Br J Pharmacol* **45**: 1–12, 1972.
46. Verbeuren TJ, Jordaens FH, Bult H and Herman AG, The endothelium inhibits the penetration of serotonin and norepinephrine in the isolated canine saphenous vein. *J Pharmacol Exp Ther* **244**: 276–282, 1988.
47. Pascual R and Bevan JA, Asymmetry of consequences of drug disposition mechanisms in the wall of the rabbit aorta. *Circ Res* **46**: 22–28, 1980.
48. Lamping KG, Marcus ML and Dole WP, Removal of the endothelium potentiates canine large coronary artery constrictor responses to 5-hydroxytryptamine *in vivo*. *Circ Res* **57**: 46–54, 1985.
49. Szwajkun K, Lamping KG and Dole WP, Role of endothelium-derived relaxing factor and prostaglandins in responses of coronary arteries to thromboxane *in vivo*. *Circ Res* **66**: 1729–1737, 1990.
50. Freiman PC, Mitchell GG, Heistad DD, Armstrong ML and Harrison DG, Atherosclerosis impairs endothelium-dependent vascular relaxation to acetylcholine and thrombin in primates. *Circ Res* **58**: 783–789, 1986.
51. Cohen RA, Zitnay KM, Haudenschild CC and Cunningham LD, Loss of selective endothelial cell vasoactive functions caused by hypercholesterolemia in pig coronary arteries. *Circ Res* **63**: 903–910, 1988.
52. Meredith IT, Anderson TJ, Uehata A, Yeung AC, Selwyn AP, Ganz P, Fung HL, Heller G, Parker JD and Cohen RA, Role of endothelium in ischemic coronary syndromes. *Am J Cardiol* **72**: 27C–32C, 1993.
53. Kalsner S, Cholinergic constriction in the general circulation and its role in coronary artery spasm. *Circ Res* **65**: 237–257, 1989.
54. Martin W, Basal release of endothelium-derived relaxing factor. In: *Relaxing and Contracting Factors. Biological and Clinical Research* (Ed. Vanhoutte PM), Chap. 8, pp. 159–178. Humana Press, Clifton, NJ, 1988.
55. Martin SE and Patterson RE, Coronary constriction due to neuropeptide Y: Alleviation with cyclooxygenase blockers. *Am J Physiol* **257** (3 Pt 2): H927–H934, 1989.
56. Cox RH and Ferrone RA, Effects of BRL-34915 on ^{86}Rb efflux and active stress in rat vascular smooth muscle. *FASEB J* **2**: A756, 1988.
57. Bassenge E and Busse R, Endothelial modulation of coronary tone. *Prog Cardiovasc Dis* **30**: 349–380, 1988.
58. Mazmanian GM, Baudet B, Pannierpoulain C, Choudat L, Dulmet E, Perrin A, Weiss M and Herve Ph, Pressor responses of rat isolated tail arteries to contractile stimulation after methylene blue treatment—Effect of adventitial versus intimal entry. *J Vasc Res* **30**: 250–256, 1993.
59. Thom S, Hughes A and Sever PS, Endothelium-dependent responses in human arteries. In: *Relaxing and Contracting Factors. Biological and Clinical Research* (Ed. Vanhoutte PM), Chap. 25, pp. 511–528. Humana Press, Clifton, NJ, 1988.
60. Rees DD, Palmer RMJ, Hodson HF and Moncada S, A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. *Br J Pharmacol* **96**: 418–424, 1989.
61. Moore PK, al-Swayeh OA, Chong NWS, Evans RA and Gibson A, L-N^G-Nitroarginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vasodilatation *in vitro*. *Br J Pharmacol* **99**: 408–412, 1990.
62. Yang Z, Richard V, von Segesser L, Bauer E, Stulz P, Turina M and Lüscher TF, Threshold concentrations of endothelin-1 potentiate contractions to norepinephrine and serotonin in human arteries. A new mechanism of vasospasm? *Circulation* **82**: 188–195, 1990.
63. Lane GE and Bove AA, The effects of cyclooxygenase inhibition on vasomotion of proximal coronary arteries with endothelial damage. *Circulation* **72**: 389–396, 1985.
64. Lefroy DC, Crake T, Uren NG, Davies GJ and Maseri A, Effect of inhibition of nitric oxide synthesis on epicardial coronary artery caliber and coronary blood flow in humans. *Circulation* **88**: 43–54, 1993.
65. Kolodgie FD, Virmani R, Rice HE and Mergner WJ, Vascular reactivity during the progression of atherosclerotic plaque. *Circ Res* **66**: 1112–1126, 1990.
66. Cohen RA, Shepherd JT and Vanhoutte PM, 5-Hydroxytryptamine can mediate endothelium-dependent relaxation of coronary arteries. *Am J Physiol* **245**: H1077–H1080, 1983.
67. Brum JM, Sufan Q, Lane G and Bove AA, Increased vasoconstrictor activity of proximal coronary arteries with endothelial damage in intact dogs. *Circulation* **70**: 1066–1073, 1984.
68. Förstermann U, Mügge A, Alheid U, Bode SM and Frölich JC, Endothelium-derived relaxing factor (EDRF): A defence mechanism against platelet aggregation and vasospasm in human coronary arteries. *Eur Heart J* **10** (Suppl F): 36–43, 1989.
69. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW and Ganz P, Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med* **317**: 1046–1051, 1986.
70. McLenachan JM, Weidinger FF, Barry J, Yeung A, Nable EG, Rocco MB and Selwyn AP, Relations between heart rate, ischemia, and drug therapy during daily life in patients with coronary artery disease. *Circulation* **83**: 1263–1270, 1991.
71. Zeiher AM, Drexler H, Saurbier B and Just H, Endothelium-mediated coronary blood flow modulation in humans. Effects of age, atherosclerosis, hypercholesterolemia, and hypertension. *J Clin Invest* **92**: 652–662, 1993.
72. Zeiher AM, Drexler H, Wollschläger H and Just H, Modulation of coronary vasomotor tone in humans. Progressive endothelial dysfunction with different early stages of coronary atherosclerosis. *Circulation* **83**: 391–401, 1991.
73. Vita JA, Treasure CB, Nabel EG, McLenachan JM, Fish RD, Yeung AC, Vekshtein VI, Selwyn AP and Ganz P, Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. *Circulation* **81**: 491–497, 1990.
74. Celermajer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J and Deanfield JE, Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* **88**: 2149–2155, 1993.
75. Winquist RJ, Endothelium-dependent relaxations in hypertensive blood vessels. In: *Relaxing and Contracting Factors. Biological and Clinical Research* (Ed. Vanhoutte PM), pp. 473–494. Humana Press, Clifton, NJ, 1988.

76. Lüscher TF, Richard V, Tschudi M, Yang ZH and Boulanger C, Endothelial control of vascular tone in large and small coronary arteries. *J Am Coll Cardiol* **15**: 519–527, 1990.
77. Komori K, Shimokawa H and Vanhoutte PM, Endothelium-dependent relaxation in response to aggregating platelets in porcine femoral veins and its modulation by diet. *Circulation* **80**: 401–409, 1989.
78. Kalsner S, Prostaglandin mediated relaxation of coronary artery strips under hypoxia. *Prostaglandins Med* **1**: 231–239, 1978.
79. Feigl EO, Coronary physiology. *Physiol Rev* **63**: 2–205, 1983.
80. Vatner SF and Murray PA, Reflex control of coronary arteries. In: *The Coronary Artery* (Ed. Kalsner S), pp. 216–238. Croom Helm, London, 1982.
81. Kalsner S (Ed.), *The Coronary Artery*. Croom Helm, London, 1982.
82. Toyo-oka T, Aizawa T, Suzuki N, Hirata Y, Miyauchi T, Shin WS, Yanagisawa M, Masaki T and Sugimoto T, Increased plasma level of endothelin-1 and coronary spasm induction in patients with vasospastic angina pectoris. *Circulation* **83**: 476–483, 1991.
83. Whittle BJR and Moncada S, The endothelin explosion: A pathophysiological reality or a biological curiosity? *Circulation* **82**: 2022–2025, 1990.
84. Gryglewski RJ, Botting RM and Vane JR, Mediators produced by the endothelial cell. *Hypertension* **12**: 530–548, 1988.
85. Stewart DJ, Kubac G, Costello KB and Cernacek P, Increased plasma endothelin-1 in the early hours after myocardial infarction. *J Am Coll Cardiol* **18**: 38–43, 1991.
86. Qiu SQ, Theroux P, Marcil M and Solymoss BC, Plasma endothelin-1 levels in stable and unstable angina. *Cardiology* **82**: 12–19, 1993.
87. Lerman A, Hildebrand FL Jr, Aarhus LL and Burnett JC Jr, Endothelin has biological actions at pathophysiological concentrations. *Circulation* **83**: 1808–1814, 1991.
88. Davies MJ, The pathological basis of angina pectoris. *Cardiovasc Drugs Ther* **3**: 249–255, 1989.
89. Hodgson JMcB, Cohen MD, Szentpetery S and Thames MD, Effects of regional α- and β-blockade on resting and hyperemic coronary blood flow in conscious, unstressed humans. *Circulation* **79**: 797–809, 1989.
90. Young MA and Vatner SF, Regulation of large coronary arteries. *Circ Res* **59**: 579–596, 1986.
91. Kalsner S, The effects of periarterial nerve activation on coronary vessel tone in an isolated and perfused slab of beef ventricle. *Can J Physiol Pharmacol* **57**: 291–297, 1979.
92. Kalsner S and Richards R, Coronary arteries of cardiac patients are hyperreactive and contain stores of amines: A mechanism for coronary spasm. *Science* **223**: 1435–1437, 1984.
93. Bush LR and Shebuski RJ, *In vivo* models of arterial thrombosis and thrombolysis. *FASEB J* **4**: 3087–3098, 1990.
94. Benedict CR, Mathew B, Rex KA, Cartwright J Jr and Sordahl LA, Correlation of plasma serotonin changes with platelet aggregation in an *in vivo* dog model of spontaneous occlusive coronary thrombus formation. *Circ Res* **58**: 58–67, 1986.
95. Nicolini FA, Nichols WW, Saldeen TGP and Mehta JL, Pathological basis of failure of concurrent glyceryl trinitrate therapy to improve efficacy of tissue-type plasminogen activator in coronary thrombosis. *Cardiovasc Res* **25**: 283–289, 1991.
96. Bertha BG and Folts JD, Inhibition of epinephrine-exacerbated coronary thrombus formation by prostacyclin in the dog. *J Lab Clin Med* **103**: 204–214, 1984.
97. Golino P, Ashton JH, Buja M, Rosolowsky M, Taylor AL, McNatt J, Campbell WB and Willerson JT, Local platelet activation causes vasoconstriction of large epicardial canine coronary arteries *in vivo*: Thromboxane A_2 and serotonin are possible mediators. *Circulation* **79**: 154–166, 1989.
98. Ashton J, Benedict CR, Fitzgerald C, Raheha S, Taylor A, Campbell WB, Buja LM and Willerson JT, Serotonin is a mediator of cyclic flow variations in stenosed canine coronary arteries. *Circulation* **73**: 572–578, 1986.
99. Ellis EF, Oelz O, Roberts CJ II, Payne NA, Sweetman BJ and Oates JA, Coronary arterial smooth muscle contraction by a substance released from platelets: Evidence that it is thromboxane A_2. *Science* **193**: 1135–1137, 1976.
100. Mullane KM, Bradley G and Moncada S, The interactions of platelet-derived mediators on isolated coronary arteries. *Eur J Pharmacol* **84**: 115–118, 1982.
101. Kalsner S, Activation of a relaxation cascade in isolated coronary arteries. *J Pharmacol Exp Ther* **261**: 209–224, 1992.
102. Ohlstein EH, Kopia GA, Zeid RL, Valocik RW, Horohonich S, Hieble JP and Wasserman MA, Effects of the thromboxane receptor antagonist SK&F 88046 in the canine, monkey and human coronary vasculature. *Prostaglandins* **36**: 69–84, 1988.
103. Maseri A, Davies G, Hackett D and Kaski JC, Coronary artery spasm and vasoconstriction. *Circulation* **81**: 1983–1990, 1990.
104. Maseri A, L'Abbate A, Baroldi G, Chierchia S, Marzilli M, Ballestra AM, Serveri S, Parodi O, Biagini A, Distante A and Pesola A, Coronary vasospasm as a possible cause of myocardial infarction. *N Engl J Med* **299**: 1271–1277, 1978.
105. Bertrand ME, La Blanche JM, Tilmant PY, Thieuleux FA, Delfarge MR, Carre G, Asseman P, Berzin B, Libersa C and Laurent JM, Frequency of provoked coronary arterial spasm in 1089 consecutive patients undergoing coronary arteriography. *Circulation* **65**: 1299–1306, 1982.
106. Allen SP, Dashwood MR, Chester AH, Tadjkarimi S, Collins M, Piper PJ and Yacub MH, Influence of atherosclerosis on the vascular reactivity of isolated human epicardial coronary arteries to leukotriene C_4. *Cardioscience* **4**: 47–54, 1993.
107. Shimokawa H and Vanhoutte PM, Angiographic demonstration of hyperconstriction induced by serotonin and aggregating platelets in porcine coronary arteries with regenerated endothelium. *J Am Coll Cardiol* **17**: 1197–1202, 1991.
108. Heistad DD, Armstrong ML, Marcus ML, Piegors DJ and Mark AL, Augmented response to vasoconstrictor stimuli in hypercholesterolemic and atherosclerotic monkeys. *Circ Res* **54**: 711–718, 1984.
109. Nagasawa K, Tomoike H, Hayashi Y, Yamada A, Yamamoto T and Nakamura M, Intramural hemorrhage and endothelial changes in atherosclerotic coronary artery after repetitive episodes of spasm in X-ray-irradiated hypercholesterolemic pigs. *Circ Res* **65**: 272–282, 1989.
110. Fukai T, Egashira K, Hata H, Numaguchi K, Ohara Y, Takahashi T, Tomoike H and Takeshita A, Serotonin-induced coronary spasm in a swine model. A minor role of defective endothelium-derived relaxing factor. *Circulation* **88**: 1922–1930, 1993.
111. Chester AH, Allen SP, Tadjkarimi S and Yacoub MH, Interaction between thromboxane A_2 and 5-hydroxytryptamine receptor subtypes in human coronary arteries. *Circulation* **87**: 874–880, 1993.
112. O'Reilly RJ, Spellberg RD and King TW, Recognition of proximal right coronary artery spasm during coronary arteriography. *Radiology* **95**: 305–309, 1970.

113. Kalsner S, Termination of agonist action on autonomic receptors. In: *Trends in Autonomic Pharmacology* (Ed. Kalsner S), Vol. 1, pp. 265–287. Urban & Schwarzenberg, Baltimore, 1979.
114. Dahms V, Prosser CL and Suzuki N, Two types of 'slow waves' in intestinal smooth muscle of cat. *J Physiol (Lond)* **392**: 51–69, 1987.
115. Ross G, Stinson E, Schroeder J and Ginsberg R, Spontaneous phasic activity of isolated human coronary arteries. *Cardiovasc Res* **24**: 659–669, 1980.
116. Biamino G and Kruckenberg P, Synchronization and conduction of excitation in the rat aorta. *Am J Physiol* **217**: 376–382, 1969.
117. Spray DC, White RL, Mazet F and Bennett MVL, Regulation of gap junctional conductance. *Am J Physiol* **248**: H753–H764, 1985.
118. Bobik A, Weissberg PL and Little PJ, Spontaneous oscillations in cytoplasmic free calcium concentration in vascular smooth muscle: A potential mechanism associated with myogenic tone. *Clin Exp Pharmacol Physiol* **15**: 281–284, 1988.
119. Kalsner S and Quillan M, Non-neurogenic relaxation to field stimulation in coronary arteries. *J Pharmacol Exp Ther* **250**: 461–469, 1989.
120. Ashida T and Blaustein MP, Regulation of cell calcium and contractility in mammalian arterial smooth muscle: The role of sodium–calcium exchange. *J Physiol (Lond)* **392**: 617–635, 1987.
121. Kalsner S, Coronary artery spasm and no spasmogens? *Med Hypotheses* **40**: 186–195, 1993.
122. Gleason MM, Medow MS and Tulenko TN, Excess membrane cholesterol alters calcium movements, cytosolic calcium levels, and membrane fluidity in arterial smooth muscle cells. *Circ Res* **69**: 216–227, 1991.
123. Strickberger SA, Russek LN and Phair RD, Evidence for increased aortic plasma membrane calcium transport caused by experimental atherosclerosis in rabbits. *Circ Res* **62**: 75–80, 1988.
124. Madden TD, Chapman D and Quinn PJ, Cholesterol modulates activity of calcium-dependent ATPase of the sarcoplasmic reticulum. *Nature* **279**: 538–541, 1979.