Role of Coronary Vasoconstriction in Ischemic Heart Disease and Search for Novel Therapeutic Targets

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Atherothrombosis has long been recognized as an important mechanism of cardiac events in ischemic heart disease, and large multicenter clinical studies have shown the benefit of antiplatelet agents, statins, β-blockers and angiotensin converting enzyme inhibitors in preventing these events. However, more recent studies have been less successful at showing incremental gains in targeting these mechanisms, suggesting that the limits of this strategy have been exploited. Coronary vasoconstriction is another important mechanism in ischemic heart disease but has received little attention and yet is a potential therapeutic target. In the current review, the reasons why coronary vasconstriction has received insufficient consideration are explored. In particular, we need to change our approach from lumping heterogeneous clinical entities together to focusing on clinically-discrete homogeneous groups with a common mechanism and thus therapeutic target. The role of coronary vasoconstriction is examined in the various ischemic syndromes (variant angina, chronic stable angina, acute coronary syndromes and syndrome X) and the underlying mechanisms discussed. Finally, in order to advance studies in this field, an innovative research strategy is proposed, including: (1) selection of paradigmatic cases for the various ischemic syndromes; (2) candidate therapeutic targets; and (3) approaches in assessing the clinical efficacy of these potential therapies. (*Circ J* 2009; **73**: 394–403)

Key Words: Coronary blood flow; Coronary circulation; Coronary vasospam; Myocardial ischemia; Vasospastic angina

T he incremental reduction of major cardiac events in recent trials using statins, angiotensin converting enzyme inhibitors or β-blockers and antithrombotic agents is getting progressively smaller because we have already exploited most of the opportunities offered by the known therapeutic targets based on the present, incomplete pathophysiological understanding of the disease. Moreover, the number of negative trials is growing, although the incidence of events in optimally treated patients remains high. Therefore, it is becoming obvious that we are not correcting some still unknown pathogenetic mechanisms of the disease.

The time has come to focus clinical biological research on phenotypically homogeneous groups of patients in order to discover novel, specific therapeutic targets "made to measure" for them. The role of coronary vasoconstriction is one of such research areas that deserve appropriate attention.

In this endeavour, clinical investigators must have inquisitive minds, rather than be conditioned by prevailing paradigms, and look for distinctive features among patients within the same clinical syndrome, which could help the identification of yet unknown pathogenetic mechanisms of disease.

The present review will first examine the reasons for the insufficient consideration given so far to the coronary vasoconstrictor components of ischemic syndromes. Then, it will summarize the available information on the role of coronary vasoconstriction in ischemic syndromes and on the actual mechanisms of vasoconstriction, taking into consideration the remarkable differences between Caucasian and Japanese observations, highlighted in the comprehensive review by Beltrame et al! Finally, it will outline possible innovative research strategies for the development of new therapeutic targets.

Insufficient Consideration for Coronary Vasoconstriction

Research on the role and mechanisms of coronary vasoconstriction was given insufficient consideration for the combination of three important components.

The Conditioning Power of Paradigms
In the late 1950s, the prevailing paradigm was that ischemic heart disease was caused by atherosclerotic coronary obstructions severe enough to determine "coronary insufficiency" with a consequent reduction of myocardial blood flow that resulted in angina when mild, and in infarction when severe. This paradigm, which originated from a widely accepted, exaggerated extrapolation of post-mortem findings and offered an apparently plausible explanation, had a profound, long-lasting, influence in 3 major areas. It conditioned for a long time clinical practice, pharmacological development and clinical research.

First, until the early 1960s, patients with acute myocardial infarction (MI) were confined to strict bed rest, and restrained from any movements for 3 weeks, in order to avoid any increase in their myocardial oxygen demand which could worsen the supposed imbalance associated

with the extreme "coronary insufficiency" responsible for the infarction.

Second, the main prerequisites of anti-ischemic drugs under development were supposed to be the ability to produce an increase in myocardial blood flow in animal models. This view was supported by the observation that nitroglycerin, which had an established anti-anginal effect, did increase coronary blood flow in animals. Yet, dipyridamole, which was the prototypic drug developed according to this hypothesis, did increase markedly myocardial blood flow, but had no demonstrable anti-anginal effect.

Third, angina could only be caused by an excessive increase in myocardial demand beyond the limited possibility of supply caused by coronary stenoses: indeed, angina at rest with preserved effort tolerance was not to be considered of cardiac origin in the classic textbook, by Friedberg[2].

Coronary artery spasm was defined "the resort of the diagnostically destitute" by Pickering as, according to pathologists, atherosclerotic coronary arteries could not constrict. Indeed, Prinzmetal, in his classic articles on variant angina[3] did not dare to use the term "spasm", but proposed an increase in coronary "tonus" at the site of a subcritical stenosis to explain spontaneous angina at rest. For a historical review on variant angina, see MacAlpin et al[4]. The anti-anginal effect of nitroglycerin, when it was shown to relieve angina without increasing coronary blood flow, was then attributed to a reduction of the cardiac workload, without any consideration of its potential coronary antivasoconstrictor effect. The hypothesis that angina could not be exclusively "secondary" to an excess increase in myocardial demand, but could also be "primary", ie, caused by a transient reduction in coronary blood flow, as a result of vasoconstriction, thrombosis or their combination, was not easily accepted[5]. Thus, the study of coronary constriction as a possible cause of angina was largely disregarded for decades, because it did not fit the prevailing, deeply engrained, paradigm.

The Elusive Nature of Coronary Vasoconstriction

In clinical practice, the possible role of coronary vasoconstriction is also disregarded, because of its very elusive nature. Epicardial coronary artery spasm and dynamic stenoses can be visualized only by performing angiography during its very transient, occasional occurrence, spontaneous or following provocative tests, and after intracoronary nitrates. They can be detected either by chance or by actively searching for them with provocative tests. These very transient dynamic alterations are in sharp contrast with atherosclerotic plaques, always visible at post-mortem and by imaging techniques, and also with thrombus that often leaves visible residues after the ischemic episodes. These easily visible alterations represent readily available, plausible culprits, commonly considered sufficient to explain by themselves ischemic events. In a busy catheterization laboratory, the workload is often so high that, with a plausible explanation available, there is no time and no inclination for further questions. The acceptance of small coronary vessels constriction is even less frequent, as small vessels are not visible angiographically[6]. In many labs, intracoronary nitrates are not routinely injected and provocative tests of spasm are only considered for patients with obvious ischemic episodes but angiographically normal coronary arteriograms. In the absence of evidence of ischemia, normal coronary angiogram often leads to the diagnosis of "non-cardiac chest pain"[6].

The Search for Common Therapeutic Targets

Grouping patients in broad clinical syndromes has become common practice for organizing large therapeutic trials and for standardizing patient management.

In order to simplify such grouping, few, easily available descriptors are used as inclusion criteria, with a consequent heterogeneity and multiplicity of the actual causes of the syndrome. This strategy is appropriate for the final stage of some syndromes, when all patients might benefit from the same treatment, regardless of the multiple pathogenetic mechanisms by which the syndromes are caused. For example, in patients with severe anemia, a blood transfusion is beneficial regardless of its actual causes. Accordingly, all patients with overt cardiac failure benefit from diuretic therapy and all patients with acute infarction benefit from prompt recanalization of the occluded coronary artery, regardless of the causes of the occlusion.

By contrast, the much more ambitious task of preventing a broad syndrome requires the precise diagnosis of its actual causes. These causes are known, easily diagnosed and specifically treated for anemia; are not always known, sometimes not easily diagnosed for cardiac failure and are only speculative for acute infarction. The actual causes of anemia could be identified, and now can be diagnosed, by searching for distinctive, specific features in medical history, red blood cells and serum assays, among patients.

The current classification of ischemic cardiac syndromes, such as acute coronary syndromes, rest angina, stable effort angina, vasospastic angina, syndrome X, is far too broad for developing specific preventive treatments, because it is based on too few, simple descriptors. Therefore, within each of these syndromes additional clinical, instrumental, biological distinctive features among patients should be identified in order to set the stage for the diagnosis of subgroups of patients with the same etiology and hence with precise therapeutic targets, like it happened for anemia. The heterogeneity of pathogenetic mechanisms within a given syndrome might result in a non-significant benefit from a drug that is beneficial in only a specific subgroup of the patients included, when their prevalence in a trial is diluted by a large number of other patients for whom the treatment is ineffective. For example, this was the case for calcium antagonists, which were found not to be effective in patients with unstable angina, probably because the trials did not include a sufficient number of patients with variant angina for whom calcium antagonists have a proven efficacy.

Role of Coronary Vasoconstriction in Ischemic Syndromes

Although the role of coronary vasoconstriction was originally discovered in "variant angina", there is convincing evidence that various types of coronary vasoconstriction play a role in major ischemic syndromes that should be investigated and appropriately treated.

Variant Angina

The hypothesis of a transient coronary occlusion was suggested by Prinzmetal in his observation of a small, but homogeneous group of patients who had a combination of unusual and very visible distinctive features: (1) they presented with angina at rest with preserved effort tolerance; (2) their anginal attacks were characterized by dramatic ischemic changes on the electrocardiograms, suggestive of transmural myocardial ischemia; and (3) some of them at

post-mortem were found to have only a subcritical coronary stenosis[3] Thus, he proposed "an increased tonus" at the site of a subcritical stenosis as the likely cause of the syndrome[7] Subsequently, variant angina was described by Cheng et al also in patients with angiographically normal coronary arteries and defined "the variant of the variant"[8] thus falsifying the paradigm that a stenosis was required for the development of spasm. However, 15 years after the publication of the Prinzmetal report, there was a prevailing scepticism at the World Congress of Cardiology in Buenos Aires in 1974, after the presentation of the angiographic findings of 3 cases of occlusive spasm abolished by nitrates by Maseri's group: most of the audience believed that spasm was an artefact induced by the catheter! Finally, the acceptance of occlusive spasm was determined by the convergent demonstration that in patients with variant angina, the ischemic attacks were not caused by an increased myocardial demand[9] were associated with massive regional defects of thallium uptake[10] and could also be caused by spasm occurring at the site of severe coronary stenoses[11] thus disproving the claims of pathologists that atherosclerotic arteries cannot constrict! Subsequently disproved also by careful post-mortem studies showing common preservation of the media surrounding stenoses[12]

Unfortunately, the lessons learned in previous generations are not strongly supported in contemporary cardiological practice in relation to variant angina. Except for Japanese cardiologists, provocative testing for coronary artery spasm is not routinely undertaken during diagnostic angiography, nor is the administration of intracoronary nitrates for the assessment of the dynamic component of an observed stenosis. Moreover, patients presenting with recurrent episodes of transient ST elevation are more likely to be considered as having recurrent thrombosis and managed with antiplatelet agents or even thrombolytics, than a diagnosis of variant angina made and vasodilator therapy instituted. Hence, clinical awareness of variant angina warrants further attention as does the elucidation of its vasospastic mechanisms, particularly in relation to differences between Japanese and Caucasians!

Chronic Stable Angina

The majority of these patients report a very variable anginal threshold, confirmed by Holter recordings, showing ischemic episodes occurring without any increase in heart rate, as well as episodes of tachycardia without evidence of ischemia[13,14] Only a few report a history of fixed threshold of effort above which they consistently develop angina, and below which they never do. This very variable ischemic threshold can only be explained by a strong modulation or residual coronary flow reserve by vasomotor tone, not only varying dynamically the lumen of the stenoses[15] but also, and probably to a greater extent, in small distal vessels[16] The pharmacological blockade of this inappropriate vasoconstriction would restore residual coronary flow reserve to its maximum value, but different drugs might be required for blocking epicardial arteries and small vessel constriction. Indeed, about 50% of the patients with stable coronary disease continue to have angina and positive exercise tests in spite of successful coronary interventional revascularization[17,18] which does not appear to have significant impact on prognosis in these patients[19]

Acute Coronary Syndromes

Contemporary theorem concerning the mechanism of ST elevation MI (STEMI) ascribes it to an atherothrombotic process involving plaque rupture with subsequent thrombotic vessel occlusion. Accordingly, for more than 20 years thrombolytics have been the mainstay therapy of STEMI, yet this strategy restores adequate patency in only 54% of affected patients[20] suggesting that other mechanisms are involved[21] Vasospastic mechanisms have been implicated with the demonstration that nitrate therapy can restore patency[22] Although vasospasm might be a consequence of vasoactive mediators (eg, serotonin) released by the atherothrombotic process, plaque rupture itself might be precipitated by coronary spasm; hence these mechanisms are strongly intertwined. However, in the presence of local coronary hyper-reactivity and mural thrombosis also intracoronary nitrates might not relieve the coronary occlusion[23]

The relative importance of coronary spasm in STEMI is further illustrated by several important studies. In Caucasians, up to a third of post-MI patients undergoing provocative spasm testing 1–6 weeks following infarction, have inducible coronary artery spasm[24,25] A parallel observation is that early administration of intravenous nitrates and verapamil (before thrombolysis) in acute STEMI patients, is associated with ST segment resolution in 36% of patients[26] A randomised, double-blind, placebo-controlled, study is currently in progress, assessing the efficacy of these antispastic agents in restoring coronary patency in STEMI patients, before primary percutaneous coronary intervention (Clinical Trial Number: ACTRN12606000232538). Moreover, racial factors must also be considered because, unlike Caucasians, over 80% of Japanese patients had inducible spasm within 2 weeks of MI[25]

Thus, similar to the above anemia analogy, multiple mechanisms (atherothrombotic and vasospasm) might result in the same clinical endpoint (STEMI) and thus specific mechanistically-targeted therapy will improve clinical outcomes. Unfortunately, unlike the well-defined causes of anemia, the underlying determinants and clinical markers of atherothrombotic and spastic mechanisms are less definitive.

In acute MI, altered vascular reactivity is not only evident in the large epicardial coronary arteries but also the microvasculature. Microvascular dysfunction in the context of acute MI has been demonstrated both in the infarct-related and non-infarct-related artery territories. Immediately following primary angioplasty, distal perfusion might be compromised despite successful ballooning/stenting of the culprit lesion[27] This no-reflow phenomenon is unresponsive to thrombolytic therapy[27] but can be effectively treated with vasodilators[28] In non-infarct-related artery territories, coronary flow responses to small vessel vasodilators has been shown to be impaired in the absence of epicardial stenoses[29] Thus, if we are to improve reperfusion therapies, attention must focus not only on the site of occlusion but also on the distal and remote micovasculature.

Syndrome X

Coronary small vessel constriction was proposed as a cause of angina in patients who typically present with stable effort angina, positive exercise test, often positive myocardial scintigraphy, but have no critical stenosis or angiographically normal coronary arteries. In addition, these patients typically develop angina with ischemic electrocardiographic changes in response to the dipyridamole and adenosine tests, which might result from coronary blood flow steal and/or from the development of critical closing

Normal vessels: Response to different stimuli
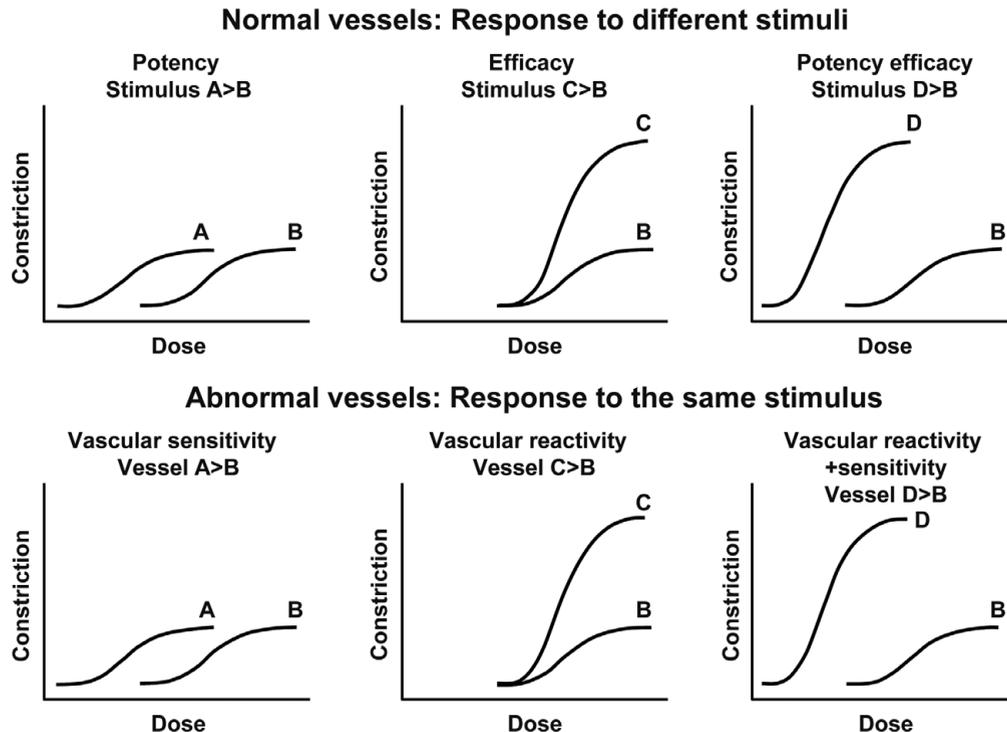
Figure 1. Vasoconstrictor stimuli and constrictor response. The vasoconstriction in response to stimuli of different potency efficacy in normal arteries is illustrated in the top panels; the response of abnormal arteries having different sensitivity and reactivity to the same stimulus is illustrated in the bottom panels. **Top left**: stimulus A is more potent than stimulus B; the same constriction is caused by a lower dose of the agonist. The maximum lumen reduction achieved is the same; therefore, the efficacy of the 2 stimuli is similar. **Top center**: stimulus C is more efficacious than B and elicits a greater maximum constriction. **Top right**: stimulus D is more potent and efficacious than stimulus B; it causes greater constriction at a lower dose. **Bottom left**: the sensitivity of vessel A is greater than that of vessel B as vessel A develops the same constriction as vessel B at lower doses of the same agonist. **Bottom center**: the reactivity of vessel C is greater than that of vessel B as vessel C develops greater constriction than vessel B in response to the same stimulus. **Bottom right**: both the reactivity and sensitivity of vessel D are greater than those of vessel B as it develops greater constriction at lower doses of agonist (modified from Ref. 29).

pressure at the end of most constricted prearteriolar vessels (see ahead). The ischemic nature of this syndrome is commonly questioned because, during angina, patients do not develop left ventricular wall motion abnormalities or myocardial lactate release. A patchily distributed microvascular dysfunction could produce small foci of ischemia dispersed in the ventricular wall, sufficient to cause pain and electrocardiographic changes but not extensive enough to cause wall motion abnormalities or detectable lactate production.[30] This possibility is strongly supported by the release of ischemic reperfusion products into the coronary sinus at the end of pacing-induced ischemia.[31] Coronary microvascular constriction or inadequate dilation might also be related to medial smooth muscle hypertrophy of pre-arterial vessels in hypertrophied myocardium.

Mechanisms of Coronary Vasoconstriction

A comprehensive examination of the mechanisms of inappropriate coronary vasoconstriction should be viewed within the general scenario of the hemodynamic control of coronary blood flow and of the development of myocardial ischemia [for a comprehensive review see: Ischemic Heart Disease – A rational basis for clinical practice and clinical research. Churchill Livingstone 1995 (Chapter 4, Page 71; Chapter 5, Page 105; Chapter 6, Page 137)]. Constriction severe enough to cause myocardial ischemia can develop

both in large epicardial coronary arteries and in small distal vessels, and it might result from 2 fundamental different pathogenetic mechanisms (**Figure 1**)[32] that require distinct therapeutic approaches:

- Strong stimuli capable of causing critical constriction also in normal vessels.
- Enhanced local constrictor response of the vascular wall to a variety of stimuli which, although do not have any significant constrictor effect on normal vessels, cause critical constriction of the affected segment.
- In addition, inadequate vasodilator response can reduce coronary flow reserve and enhance the effect of constrictor stimuli.

This overview is intended to provide useful clues for developing novel clinical and biological pathogenetic research strategies, designed to identify new specific therapeutic targets.

Hemodynamic Regulation of Coronary Blood Flow and Determinants of Ischemia

Epicardial coronary arteries down to approximately $500\,\mu m$ offer negligible resistance to flow and distal pre-arteriolar vessel down to approximately $100\,\mu m$ contribute only 10–20% of total coronary resistance. Thus, normally resistance and hence flow are controlled by arterioles, defined in functional terms as the site of the metabolic control of coronary flow. Arteriolar tone is controlled by the intersti-

Table 1. Frequency of Inducible Spasm in Caucasian and Japanese MI Patients Compared With Stable CAD Patients

Study	Patient race	Provocative agent	MI		Stable CAD	
			n	Spasm	n	Spasm
Recent MI						
Bertrand[50]	Caucasian	Methylergonovine	131	21%	353	9%
Pristipino[25]	Caucasian	Acetylcholine	19	37%		
Pristipino[25]	Japanese	Acetylcholine	15	80%		
Okumura[51]	Japanese	Acetylcholine	16	69%	16	21%
Remote MI (>6 weeks)						
Bertrand[24]	Caucasian	Methylergonovine	64	6%	248	1%
Nosaka[52]	Japanese	Ergonovine	398	23%	648	1%

MI, myocardial infarct; CAD, coronary artery disease (from Ref. 1).

tial concentration of myocardial vasodilator metabolites, a key mechanism for matching flow demand and supply. Arteriolar tone is near maximal in basal conditions and decreases progressively with the interstitial accumulation of vasodilator metabolites, providing very large coronary flow reserve that allows flow to increase linearly as long as perfusion pressure at their origin remains constant.

The Key Role of Perfusion Pressure   In the presence of a critical stenosis, which reduces coronary perfusion pressure at the origin of arterioles, flow is maintained constant by the progressive increase in vasodilator metabolites in the myocardial interstitium. The consequent arteriolar dilation compensates for the reduction in their perfusion pressure at the expense of a reduction in coronary flow reserve, until coronary flow reserve is exhausted.

When perfusion pressure at the origin of the arterioles is so low that it becomes insufficient to maintain adequate myocardial perfusion, in spite of maximal arteriolar dilation, ischemia eventually develops. Subendocardial layers of the left ventricular wall are more vulnerable to ischemia than subepicardial ones, because of their higher extravascular tissue pressure but when a large coronary artery is suddenly occluded, arteriolar perfusion pressure becomes so low that ischemia is transmural.

Arteriolar perfusion pressure can decrease critically also in the absence of a severe epicardial coronary artery obstruction, when a critical constriction occurs at the level of pre-arteriolar vessels that connect epicardial arteries to arterioles. When increased flow causes a large pressure drop along constricted prearteriolar vessels, distending pressure at their distal end might no longer be sufficient to maintain the vessel open and critical closing pressure develops.

When pre-arteriolar vessels are all equally constricted in a segment of the ventricular wall, the distribution of ischemia is similar to that produced by obstruction of a large parent artery. However, the extent of constriction might not involve uniformly all prearteriolar vessels and, when only few, sparse pre-arteriolar vessels are constricted, ischemia in the vascular bed perfused by each of them might be sufficient to cause pain and electrocardiogram changes, but not extensive enough to produce detectable wall motion abnormalities, as it might be the case in patients with syndrome X.

Occasional, Prolonged and Persistent Constriction, Critical and Subcritical   When critical epicardial or small distal vessels constriction is occasional and transient, it manifests clinically with angina at rest, with ST-segment elevation if transmural, with ST-segment depression if subendocardial, and with wall motion abnormalities if extensive enough. When prolonged, it might cause infarction or stunning. The constriction might be subcritical but persistent, causing only a variable reduction in coronary flow reserve with ischemia on effort and a variable exercise tolerance, sometimes with occasional exacerbations causing episodes of angina at rest.

In all ischemic syndromes, these temporal patterns of coronary constriction can result from strong constrictor stimuli, from increased responsiveness to constrictor stimuli and from a deficient vasodilator response to vasodilator stimuli. The latter condition cannot offer a plausible explanation for ischemic episodes not associated with increased myocardial oxygen demand as it can only reduce coronary flow reserve. However, it might also increase the effect of constrictor stimuli [see Ischemic Heart Disease – A rational basis for clinical practice and clinical research. Churchill Livingston, 1995 (Chapter 5, Page 105 and Chapter 9, Page 237)].

Strong Constrictor Stimuli

The existence of constrictor stimuli capable of producing massive myocardial ischemia without detectable constriction of large epicardial coronary arteries was obtained in humans, dogs and rabbits. In addition, a variety of constrictor stimuli were shown to produce coronary small vessel constriction with or without ischemia.

In patients, the intracoronary injection of neuropeptide Y (NPY), a neurotransmitter co-secreted with noradrenalin from adrenergic nerve endings, was shown to cause nearly complete arrest of contrast medium progression along large epicardial coronary arteries, with evidence of severe ischemia, reverted by large doses of intracoronary nitrates[33] Interestingly, in animals, receptors for NPY were only found in coronary arterial vessels less than 500 μm in diameter.

In dogs, endothelin, a peptide produced by activated endothelium, was shown to cause a dose-dependent reduction in coronary flow, eventually leading to massive transmural ischemia without constriction of large epicardial arteries[34]

In rabbits, fLMP, a vasoactive peptide released by activated neutrophils, was shown to cause massive ischemia[35]

Finally, neural stimuli were shown to reduce coronary flow in dogs[36] and vasoactive agents, such as high-doses of acetylcholine[37,38] and serotonin[39] were shown to cause small coronary vessel constriction in humans [ibid Chapter 5, Page 105].

Thus, a large variety of constrictor stimuli can modulate coronary flow reserve, might cause acute myocardial ischemia or influence its severity and duration, also in the absence of atherosclerotic lesions, by acting on small distal vessels [see Ischemic Heart Disease – A rational basis for clinical practice and clinical research. Churchill Livingston,

Table 2. Vasoconstrictor Responses of Non-Spastic Segments

Study	Provocative agent	Control group		Variant/vasospastic angina	
		n	% Vasoconstriction	n	% Vasoconstriction
Caucasian patients					
Freedman et al[46]	Ergonovine	21	17±12	11	20±14
Hackett et al[55]*	Ergonovine	9	16±6	6	20±14
Kaski et al[56]*	Ergonovine	9	10±6	13	15±11
Japanese patients					
Hoshio et al[51]	Ergonovine	35	8±6	30	24±11**
Kuga et al[52]*,***	Ergonovine	24	27±10	20	38±14†,††
Okumura et al[53]	Acetylcholine	20	12±12	36	28±17†
Kugiyama et al[50]***	Acetylcholine	25	12±12	20	50±39†††
		12	10±10	9	46±31†††

*Study patients enrolled conform to variant angina definition. **$P<0.01$ vs control group. ***Angiographically normal coronary arteries. †$P<0.05$ vs control group. ††Non-spastic segments include segments adjacent to spastic site and from non-spastic arteries. †††$P<0.001$ vs control group. Vasoconstriction data presented as mean±SD (from Ref. 1).

1995 (Chapter 9, Page 237)].

Enhanced Local Constrictor Response

As no stimuli were found capable of causing occlusive spasm of epicardial coronary arteries, it appears necessary to assume the presence of local alterations of the vascular wall that determine the development of occlusive spasm in variant angina and possibly in some patients with acute coronary syndromes [see Ischemic Heart Disease–A rational basis for clinical practice and clinical research. Churchill Livingston, 1995 (Chapter 19, Page 559)].

In paradigmatic patients with variant angina, these alterations are typically segmental and appear postreceptoral. Indeed, occlusive spasm was shown to develop in response to a variety of stimuli acting on different receptors[40] not only ergonovine and acetylcholine but also histamine[41] dopamine[42] α-adrenergic stimulation[42,43] serotonin[39] and by increasing blood pH up to 7.7[44] This conclusion is consistent with the observations obtained by Shimokawa in porcine models[45,46] (see ahead). Clues to a local nature of these alterations are provided by the documented recurrence of spasm in the same coronary segment over weeks and months, and by the provocation of spasm by the intracoronary injection of ergonovine[47] and acetylcholine[48] typically only in 1 segment, although all the arteries are also equally exposed to the drug. This local vascular alteration can also be multifocal and sometimes diffuse, particularly in Japanese patients. Moreover, in Japanese patients, the response to constrictor stimuli that cause occlusive segmental spasm extends, although to a much lesser extent, also to adjacent segments, a phenomenon not observed in Caucasian patients[1] (see Table 2 in Ref. 1). A similar post-receptoral enhanced responsiveness to constrictor stimuli, which is recognized in patients with the typical features of variant angina, appears to be prevalent also in the very early days post acute MI, because of the common provocation of occlusive spasm not only by ergonovine[24] or acetylcholine[25] but also by serotonin[49] The combination of a local enhanced constrictor responsiveness with the multiple constrictor stimuli released by thrombus could be an important determinant of coronary occlusion and of its persistence.

An enhanced constrictor responsiveness appears to play a role also in patients with chronic stable angina as they were shown to develop coronary stenosis constriction in response to the acetylcholine test but occlusive spasm in these patients develops very seldom (**Table 1**)[1,24,25,50–52]

Finally, an increased coronary microvascular constrictor responsiveness was also shown in some patients with syndrome X[38]

In patients with variant angina, the intensity of responsiveness varies in time, not only waxing and waning over periods of days, weeks and months, but also in some cases, with a circadian distribution as indicated by the varying incidence of anginal attacks and of the response to provocative tests[53] The enhanced responsiveness in many patients disappears completely after weeks or months, as shown by the absence of angina and by the negativity of provocative tests[54,55] Also following acute infarction, the incidence of positive tests decreases markedly with time. Thus transient functional alterations, independent from the resisting atherosclerotic background, should be postulated. These might be multifocal or diffuse.

The mechanisms of enhanced constrictor response might be multiple and are not necessarily the same in all ischemic syndromes, but might have some final common key molecular mechanisms.

Inadequate Dilator Response

The presence of atherosclerosis, with its associated endothelial dysfunction and defective production of nitric oxide, is commonly held responsible by itself, for coronary constriction, a widely accepted paradigm, which strongly conditions lines of research. This view is supported by the constrictor response to acetylcholine and by the defective flow mediated vasodilation of atherosclerotic segments. However, atherosclerosis is far too common to account, by itself, for occasional episodes of vasoconstriction, which occur only in a small minority of patients with coronary atherosclerosis and are often limited to a period of days, weeks or months, yet they are totally absent in most patients with very extensive coronary disease. In addition, the constrictor effect of acetylcholine can also be related to alterations of the media with a displacement to the left of the dose response curve of the smooth muscle to the direct constrictor effect of the drug (**Figure 1**). This possibility is supported by the finding that substance P, an endothelial dependent vasodilator, can dilate coronary stenoses in stable patients[56] indicative of a sufficiently preserved endothelial function.

Therefore, with an open minded view, it seems reasonable to assume, that endothelial dysfunction can reduce coronary flow reserve and thus account for effort angina. It might play a contributory rather than a causal role in coronary constriction and angina at rest because the multiple constrictor mechanisms involved might differ among major cardiac

ischemic syndromes and are likely to have a greater effect in the presence of endothelial dysfunction.

Innovative Research Strategies

The reduction of smooth muscle tone in the whole vascular bed, in order to oppose a localized coronary constriction, appears a suboptimal therapeutic strategy. Available vasodilators administered systemically are not always sufficient for the complete control of severe variant angina[57,58] and appear to have a smaller dilator effect on pre-arteriolar vessels than on proximal coronary arteries. Complete cardiac denervation,[59] serotonin[60,61] and thromboxane A2[62] blockade did not abolish episodes of coronary spasm. Therefore, the time has come to try and identify novel specific therapeutic targets against the strong coronary constrictor stimuli and against key molecular components of the enhanced constrictor responsiveness that typically characterizes these large and small vasomotor disorders.

However, the causes of coronary constriction in each major ischemic syndrome might be multiple, like the causes of anemia. Novel unsuspected pathogenetic component can only be identified by careful clinical research and novel working hypotheses thus developed should be tested in animal models for investigating suitable therapeutic targets.

Selection of Paradigmatic Cases

As the causes of the same ischemic syndrome might be multiple, cardiologists should stop being "lumpers", searching for a single common causal denominator for the widest possible spectrum of patients to be treated with the same drug in the wake of the current "one size fits all" approach. They should become "splitters" with an open, inquisitive mind searching for distinguishing features in clinical history, results of tests, biological parameters and angiographic findings, which could suggest distinct mechanistic hypotheses.

The first step is the selection of paradigmatic homogeneous groups of patients who are most likely to have the same pathogenetic components. These patients should be followed according to standardized research protocols, preferably long term considering the waxing and waning phases of their disease, in order to increase their homogeneity. In addition, the findings should be confirmed in parallel studies, possibly multi-ethnic.

In the search for the candidate mechanisms of coronary constriction, the investigation of extreme, homogeneous subgroups of patients who present with the most obvious distinguishing features is most likely to reveal useful clues which can then be subsequently investigated.

A limited number of homogeneous, paradigmatic cases is sufficient for making observations suggestive of new working hypotheses, but a sufficient number of cases can be collected in a reasonable time only by networks of centers interested in clinical research.

Four examples will illustrate sets of descriptive features which might represent starting points for the identification of homogenous, paradigmatic cases within major ischemic syndromes.

(1) Among patients with variant angina, the mechanism of occlusive spasm should be initially investigated in those with all the following distinguishing features: (a) frequent anginal episodes; (b) characterized by ST segment elevation on Holter recordings; (c) negative exercise stress test performed after sublingual nitrates; and (d) occlusive spasm at angiography.

Within this group, additional distinguishing features might provide clues of specific pathogenetic components in selected subgroups, such as those with obvious circadian distribution of the ischemic episodes, those without angiographic evidence of coronary stenoses at the spastic site, and those with multifocal or diffuse spasm.

(2) Among patients with chronic stable angina, the mechanisms of coronary constrictor stimuli should be initially investigated in those with all the following distinguished features: (a) very variable ischemic threshold; (b) no previous history of acute coronary syndromes; (c) no previous revascularization procedures; (d) single-vessel disease; and (e) positive exercise test and positive Holter with episodes occurring at very variable heart rate.

Within this selected paradigmatic group, the mechanisms of small vessel constriction should be investigated in those patients in whom the same symptoms and findings persist after successful coronary revascularization.

(3) Among patients with acute coronary syndromes, the mechanisms of coronary small vessel constriction should be investigated in those with the "no reflow phenomenon" after successful recanalization of the occluded culprit coronary artery by primary percutaneous transluminal coronary angioplasty and all of the following distinguishing features: (a) onset of symptoms <3 h and persistent ST segment elevation; (b) no or only minor pathological Q waves; (c) no or only minor elevation of troponins; and (d) no history of previous coronary disease.

Within this group, those with features a, b, c, d, who are found to have normal coronary arteries and apical ballooning could provide clues on the pathogenetic mechanisms of the Tako-Tsubo syndrome.

(4) Among patients with syndrome X, the mechanisms of coronary microvascular constriction should be investigated in those with all the following distinctive features: (a) a history of effort angina with pain persisting >5 min after the termination of the effort; (b) positive exercise test and Holter monitoring; (c) positive dipyridamole test for angina and ST segment depression without regional left ventricular wall motion abnormalities detected by echocardiography; (d) angiographically normal coronary arteries; and (e) negative tests for epicardial coronary spasm. Those who also report prolonged episodes of angina at rest, requiring emergency hospital admission[63] and those with myocardial hypertrophy[64] might belong to separate subgroups.

Candidate Therapeutic Targets

The strategy for the identification of potential therapeutic targets differs for the various mechanisms of coronary constriction and innovation requires a rigorous examination of apparently plausible explanations offered by prevailing paradigms.

Defective Endothelial Function   The molecular mechanisms of defective endothelial function and more broadly, of abnormal vasomotor reactivity related to atherosclerosis can be studied in animal models, with the aim of developing specific therapeutic targets to correct them.

However, a novel avenue of research would be the search for the protective mechanism which prevent many patients with extensive coronary atherosclerosis from having clinical manifestation of coronary constriction.

Asking new questions challenges established paradigms, but could open up new avenues in a rather stale research field.

Strong Constrictor Stimuli   Clues of a possible role of

NPY are available for Tako-Tsubo syndrome, in which elevated circulating blood levels were reported[65] The role of NPY should also be investigated in patients with syndrome X, particularly in the subgroup presenting with prolonged anginal episodes at rest that require emergency hospital admission. Finally, it could also be investigated in patients with acute infarction and no reflow phenomenon described above, together with the role of fLMP and of endothelin.

The role of strong constrictor stimuli should be initially supported by measurements of coronary arterio-venous differences, possibly during ischemic episodes, and subsequently proven by their inhibition by specific blockers in the same patients in whom they were shown to be released into the coronary sinus.

Enhanced Constrictor Responsiveness   The molecular mechanisms of the enhanced smooth muscle responsiveness, shown in variant angina and some cases of acute MI can only be studied in animal models, because of the difficulty to obtain tissue specimens of human coronary spastic segments. The number of post-mortem studies in patients who died of occlusive coronary artery spasm is limited[66–73] and no molecular studies were performed. Convincing porcine models, mimicking characteristic features of human coronary spasm, ie, the response to agents acting on different receptors, were developed by Shimokawa's group, using atherogenic or inflammatory stimuli[45,46] In both models, a key alteration responsible for the enhanced constrictor responsiveness of the smooth muscle was found to be mediated by Rho-kinase related pathways, which regulate the phosphorylation of myosin light chains. In turn, the enhanced myosin phorphorylation, appears to be related to an upregulation of Rho-kinase. Fasudil, a specific inhibitor of Rho-kinase, was indeed found to inhibit spasm not only in the porcine model[74] but also in patients with vasospastic angina,[75] stable effort angina[76] and in two-thirds of patients with syndrome X[77] The effectiveness of this drug shows a key pathogenetic role of this molecular mechanism of coronary smooth muscle hyper-responsiveness, which might characterize patients with distinct ischemic syndromes. This represents the first example of the potential of innovative research, for identifying key effective therapeutic targets. This line of research should be pursued as this therapeutic target might not be equally critical for all patients, within and among the various ischemic syndromes.

Assessment of Clinical Efficacy

The convincing proof of the efficacy of a drug which opposes a specific molecular mechanism of disease, identifies patients with a final common pathogenetic pathway. However, the example of iron deficiency in anemia describes the possibility of a more complex scenario because its treatment varies according to the actual cause of the deficiency itself.

A convincing proof of efficacy in individual patients can be obtained in pilot trials of paradigmatic patients, with very frequent anginal attacks and positive provocative tests, with a double cross-over design[78,79] to assess the consistency and reproducibility of the efficacy of the drug in the same patient. In such studies, a consistent lack of efficacy of the drug, at maximal dosage, would identify a subgroup of patients in whom to search for alternative therapeutic targets.

Perspectives

The identification of a Rho-kinase inhibitor as a novel

therapeutic target for coronary spasm and vasoconstriction represents a rational innovative, breakthrough[80–83] Indeed it opens the way to pharmacological research for the development of drugs, which oppose specific mechanism of coronary constriction that could replace presently available systemic vasodilator drugs, and find broad fields of application within major ischemic syndromes.

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