MEDICAL GRAND ROUNDS

University of Texas Health Science Center

Dallas, Texas

January 29, 1981

Prostaglandins and the Cardiovascular System

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I. CASE REPORT

O.C. is presently a 32 year old black man who was in good health until February, 1979, when he was hospitalized with severe chest pain of several hours duration. Electrocardiograms, cardiac enzymes, and pyrophosphate scintigraphy demonstrated an acute inferior myocardial infarction. He recovered from his infarction without complications, but soon after discharge, he began to have limiting angina pectoris and, therefore, was readmitted for cardiac catheterization. His intracardiac pressures were normal; his left ventriculogram revealed a wall motion abnormality consistent with an old inferior infarction; and his coronary arteries were angiographically normal. Following baseline selective coronary arteriography, he was given intravenous ergonovine maleate in an attempt to elicit coronary arterial spasm. With a total of 0.40 mg of ergonovine administered in divided doses, he developed no chest pain, no electrocardiographic alterations, and no angiographic evidence of coronary arterial spasm, and he was discharged on no medications.

The patient did well for several months, and in July, 1979, he had a negative maximum exercise tolerance test. In January, 1980, he began to have chest pain and was hospitalized with severe pain accompanied by minimal transient anterior ST segment elevation. The ST segment elevation resolved, and he did not evolve a myocardial infarction. A repeat catheterization was performed approximately 48 hours after the patient's most recent episode of chest pain. It included (a) measurement of aortic and coronary sinus thromboxane and prostacyclin metabolite concentrations and (b) selective coronary arteriography before and after ergonovine administration. The coronary arteriography continued to show angiographically-normal coronary arteries, and there was no response (clinical, electrocardiographic, or angiographic) to a total of 1.15 mg of ergonovine maleate in divided doses. With hospitalization, the patient's chest pain resolved, and he was discharged on no medications without a clear diagnosis. Subsequent analysis revealed that the patient had a 20-fold increase of thromboxane B_2 in coronary sinus blood in comparison to aortic blood, whereas normal individuals have roughly the same concentration of thromboxane B_2 in both aortic and coronary sinus blood.

Three months later (in March, 1980), O.C. was hospitalized with an acute anterior myocardial infarction. He had persistent chest pain post-infarction, ultimately requiring intravenous nitroglycerin for relief. Subsequently, he became pain-free, and approximately 6 weeks later a limited procedure was done in the catheterization laboratory, including (a) provocation with ergonovine maleate and cold pressor, and (b) sampling of aortic and coronary sinus blood for thromboxane and prostacyclin metabolites. Ergonovine provocation again failed to induce coronary arterial spasm, and there were no electrocardiographic changes in response to cold pressor stress. In contrast to January, 1980, there was no transcardiac increase in thromboxane B_2. Despite the lack of a firm

diagnosis, the patient was begun on oral verapamil and low-dose aspirin. Over the past 8 months, he has been pain-free, without further coronary events.

In summary, O.C. is a relatively young man with 2 well-documented myocardial infarctions, angiographically-normal coronary arteries (on two separate catheterization studies), and a consistently negative response to all attempts to provoke coronary arterial spasm, including large doses of intravenous ergonovine maleate and cold pressor stimulation. On one occasion he was found to have a greatly elevated transcardiac concentration of thromboxane. Finally, he has responded well to empiric therapy with verapamil (a calcium antagonist) and low-dose aspirin.

Over the past 10-15 years our knowledge of ischemic heart disease has mushroomed. First, we have acquired a detailed understanding of myocardial ischemia and infarction at a cellular and subcellular level. Second, we have documented precisely the natural history of ischemic heart disease and its various clinical manifestations. Third, we have succeeded in identifying patients with ischemia and/or infarction who are at especially high risk for recurrent infarction, congestive heart failure, and/or sudden death. Fourth, we have made substantial advances in both the medical and the surgical therapy of acute and chronic ischemic heart disease. Despite these sizeable steps forward, we are still painfully ignorant about the pathophysiologic events which (a) lead to the development and progression of the atherosclerotic process and (b) lead to the appearance of important and often catastrophic events in patients with underlying coronary artery disease. In simple terms, we are still uncertain what factors convert stable to unstable angina pectoris and, in turn, angina pectoris to acute myocardial infarction. Is coronary arterial spasm operative in some of these patients? Are platelet thrombi important in making the stable patient suddenly unstable? Or, do unstable angina pectoris and acute myocardial infarction occur because of a simple and mechanical event, such as sudden hemorrhage into an atherosclerotic plaque with resultant coronary arterial occlusion?

Of the humoral mediators which may be important in the pathophysiology of catastrophic events in ischemic heart disease, prostaglandins are an attractive possibility, and, as a result, you will read of these substances many times during the next several years. Today, therefore, I want to discuss with you the possible role of prostaglandins in patients with the various manifestations of ischemic heart disease. We will not discuss the pathophysiologic or pharmacologic role of prostaglandins in infants with congenital heart disease.

II. FUNDAMENTALS OF CARDIOACTIVE PROSTAGLANDINS

A. Metabolic Pathways Prostaglandins are not stored

in the body. Rather, they are synthesized in response to a variety of stimuli, after which they are active locally and then rapidly degraded. Arachidonic acid, a polyunsaturated fatty acid that is the major prostaglandin precursor, is obtained directly from dietary meat and indirectly from the essential fatty acid, linoleic acid, which is found in dietary vegetables (1, 2). Arachidonic acid is transported in blood bound to albumin and is encorporated as a structural component of phospholipids in cell membranes and other subcellular structures of all tissues of the body (3). In response to a variety of mechanical and humoral stimuli, a series of phospholipases is activated, probably through the mobilization of calcium, which enzymatically cleaves arachidonic acid (principally from phosphatidyl choline and phosphatidyl inositol)(4). Arachidonic acid is liberated and becomes available for further metabolism via two separate pathways. The first is centered on the lipoxygenase enzymes. It results in the formation of a group of compounds known as the hydroperoxy-arachidonic acids, several of which are responsible for Slow Reacting Substance of Anaphylaxis (SRS-A) activity (5). The second pathway involves the enzyme cyclooxygenase (also called prostaglandin synthetase), which results in the formation of the prostaglandins.

Presently, little is known about the activity and function of the products of the lipoxygenase pathway. Many therapeutic interventions are designed to block the cyclooxygenase pathway, thus providing more substrate for the lipoxygenase pathway. As a result, the products of this relatively unknown pathway probably will occupy our attention in the future. For the present, however, most of our attention is directed at the cyclooxygenase pathway and its products, and it is this pathway around which our discussion today will be centered.

Figure 1 (page 6) displays the pathways by which arachidonic acid is metabolized to the various prostaglandins. Arachidonic acid is converted by the enxyme cyclooxygenase to PGG_2 and PGH_2, which are known collectively as cyclic endoperoxides (6). In turn, these unstable intermediary substances are converted to a series of prostaglandins (7). The exact end-product of arachidonic acid metabolism is determined by the specific cell type in which the cyclic endoperoxides become available: in platelets, these substances are converted by the enzyme thromboxane synthetase to thromboxane $(Tx)A_2$; in turn, in vascular endothelium, they are converted by prostacyclin synthetase to prostacyclin (PGI_2). TxA_2 and PGI_2 are the predominant prostaglandins synthesized within the microcirculation of the heart, and, therefore, they will serve as the center of our discussion today.

B. Thromboxane A_2 TxA_2 is the most powerful endogenous constrictor of arteries and promoter of platelet aggregation (8). It is synthesized and released by actively aggregating platelets. At physiologic temperature and pH, TxA_2 is extremely unstable, having a serum half-life of

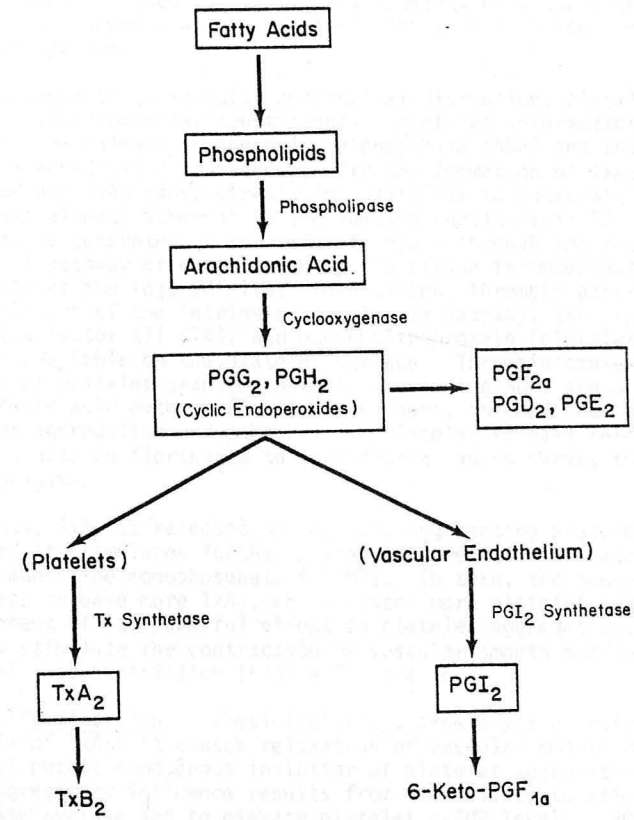

Figure 1 The pathway by which arachidonic acid is formed and then metabolized to the various prostaglandins. In response to a variety of stimuli, a series of phospholipases is activated, which enzymatically cleaves arachidonic acid. Subsequently, arachidonic acid is converted by the enzyme cyclooxygenase to PGG_2 and PGH_2, which are known collectively as cyclic endoperoxides. In turn, these substances are converted to a series of prostaglandins ($PGF_{2\alpha}$, PGD_2, PGE_2) as well as to thromboxane and prostacyclin. Specifically, PGG_2 and PGH_2 are converted in platelets by thromboxane (Tx) synthetase to TxA_2, a powerful vasoconstrictor and aggregator of platelets. Alternatively, the cyclic endoperoxides are converted in vascular endothelium by prostacyclin (PGI_2) synthetase to PGI_2, a powerful vasodilator and inhibitor of platelet aggregation. Finally, both TxA_2 and PGI_2 are unstable and are quickly converted to inactive metabolites, TxB_2 and 6-keto-$PGF_{1\alpha}$, respectively.

approximately 30 seconds (1). It is spontaneously converted to TxB_2, an inactive compound with sufficient stability to allow its collection and quantitation.

In response to vascular endothelial disruption, platelets interact with the subendothelial constituents. Platelet interaction with collagen leads to the release of adenosine diphosphate (ADP) and the activation of the arachidonic acid pathway, with the formation of TxA_2 (9-12). Both ADP and TxA_2 cause circulating platelets to aggregate with the platelets already adherent to the damaged endothelium (13). Subsequently, thrombin is generated at the site of injury through the activation of the extrinsic pathway of coagulation by the tissue thromboplastin that becomes available at the injured site. In addition, thrombin generation is promoted by activation of the intrinsic coagulation pathway: the exposed collagen activates factor XII (14), and phospholipoprotein (platelet factor 3) becomes available on the platelet surface. Thrombin causes the further release of platelet granule contents (including ADP) and activates the arachidonic acid pathway (4, 15). In short, thrombin causes further platelet aggregation and enhances the platelet release reaction. Finally, thrombin acts on fibrinogen to form fibrin, which serves to stabilize the aggregate.

Thus, TxA_2 is released by actively aggregating platelets. Upon its release, it stimulates further platelet aggregation by reducing platelet cyclic-adenosine monophosphate (c-AMP). In turn, the newly aggregated platelets release more TxA_2, which causes more platelet aggregation. Independent of its powerful effect on platelet aggregation, TxA_2 also acts to stimulate the contraction of vascular smooth muscle, leading to arterial vasoconstriction (Figure 2, page 8).

C. Prostacyclin Physiologically, prostacyclin (PGI_2) is the exact opposite of TxA_2: it causes relaxation of vascular smooth muscle and is the most potent endogenous inhibitor of platelet aggregation (6, 16). Its anti-aggregatory influence results from its ability to stimulate platelet adenylate cyclase and to elevate platelet c-AMP levels. PGI_2 is produced via the arachidonic acid cascade within vascular endothelial cells. The enzyme responsible for its production--prostacyclin synthetase--is most highly concentrated in the vascular endothelial cells nearest the vessel lumen, and its activity progressively diminishes toward the adventitial surface (17). Similar to TxA_2, PGI_2 has a very short serum half-life at body temperature and pH (roughly 2-3 minutes), after which it is metabolized to several substances, the most abundant of which in plasma is 6-keto-$PGF_{1\alpha}$, a stable and inactive compound.

D. Thromboxane-Prostacyclin "Balance" TxA_2 and PGI_2 are synthesized from the same "parent" compound (arachidonic acid). Despite their "common ancestry," they are completely opposite in physiologic properties. TxA_2, synthesized and released by platelets during aggregation, is a powerful constrictor of arteries and aggregator of platelets. In contrast, PGI_2, synthesized and released by vascular endothelium, is an equally powerful

FIGURE 2

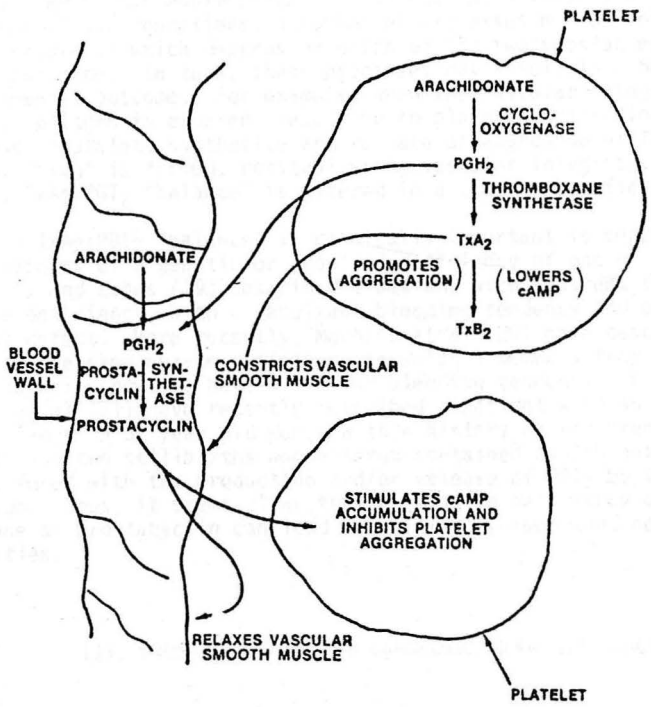

Figure 2 A schematic representation of thromboxane:prostacyclin balance within the circulation. On the left is the blood vessel wall, on the right the lumen of the vessel, within which there are 2 platelets. In the blood vessel wall, arachidonic acid (arachidonate) is converted to PGH_2 (a cyclic endoperoxide) and then to prostacyclin, which (a) relaxes vascular smooth muscle and (b) stimulates cAMP accumulation in platelets and, therefore, inhibits platelet aggregation. In contrast, in the platelet, arachidonate is converted to PGH_2 and then to TxA_2, which (a) constricts vascular smooth muscle and (b) lowers cAMP accumulation in platelets, thus promoting aggregation. From Reference #18.

dilator of arteries and inhibitor of platelet aggregation. Numerous investigators have hypothesized that normal vascular integrity and function depend on an intricate balance between TxA_2 and PGI_2 (18) (Figure 2). When this equilibrium is disrupted by a variety of physiologic or pathologic conditions, a number of processes may be initiated, the exact nature of which depends on which of the two substances is in relative abundance. In turn, these processes may result in a beneficial or a detrimental outcome. For example, when the vascular integrity is disrupted, collagen is exposed, resulting in platelet activation. In the process, platelets synthesize and release an abundance of TxA_2, and a platelet "plug" is formed, reestablishing vascular integrity. Temporarily, therefore, TxA_2:PGI_2 "balance" is altered in a clearly beneficial way.

That a TxA_2:PGI_2 "balance" is clinically important is supported by isolated reports of a genetic or acquired deficiency of one or the other. First, Weiss and Lages (19) described a patient with apparent thromboxane synthetase deficiency, with a resultant bleeding tendency and platelet functional defect. More recently, Machin, et al (20) have described a family with the same enzyme deficiency in which 3 members from 3 successive generations were shown to have a similar bleeding tendency. Conversely, Carreras, et al (21) have recently described a patient with an acquired PGI_2 deficiency: a 31 year old woman with a history of recurrent arterial thromboses and two stillbirths whose serum contained an IgG antibody that interfered with the production and/or release of PGI_2 by vascular endothelium. Thus, it seems clear that a relative deficiency of either thromboxane or prostacyclin can lead to clinically-important hemostatic abnormalities.

III. PROSTAGLANDINS AND CARDIOVASCULAR RISK FACTORS

The development and progression of peripheral, cerebral, and coronary atherosclerotic disease are intimately associated with a series of risk factors. Although there are no definitive in vivo data that link the various risk factors with prostaglandins, there is an abundance of circumstantial evidence to suggest that these risk factors may exert their effects, at least in part, through alterations in the production and action of prostaglandins.

A. Atherosclerosis/Hyperlipidemia Studies in rabbits in which atherosclerosis has been induced by an atherogenic diet have demonstrated that the atherosclerotic process reduces the ability of the coronary arteries and aorta to synthesize and to release PGI_2 (22, 23). This reduced capacity to synthesize PGI_2 may be due to the chronic inhibition of PGI_2 synthetase by lipid peroxides, substances known to be generated

excessively in atherosclerosis (23). Despite the demonstration that PGI_2 production is altered in experimental atherosclerosis, the atheromatous lesions produced in these animals differ morphologically from those which develop much more slowly in man. For this reason, recent attempts have been made to examine the interaction of prostaglandins and atherosclerosis in human tissue. Using human vascular endothelium in vitro, Moncada, et al (24) have demonstrated that lipid peroxides formed from dietary fatty acids and found in human atherosclerotic plaques selectively inhibit PGI_2 formation. Subsequent in vitro studies with human vascular tissue have confirmed the diminished ability of atherosclerotic tissue to produce PGI_2 (25, 26). In short, in tissue from both experimental animals and man, the atherosclerotic process is accompanied by a reduced capability of PGI_2 generation and release.

Conversely, the atherosclerotic process and hyperlipidemia may adversely affect the thromboxane:prostacyclin "balance" by stimulating the production and release of excess amounts of thromboxane. Platelets from atherosclerotic rabbits produce more thromboxane in response to arachidonic acid than do those from non-atherosclerotic rabbits (27); furthermore, these platelets are more sensitive to the proaggregatory actions of collagen and ADP (28). Since these animals develop high serum lipid levels during feeding with an atherogenic diet, these platelet abnormalities may be due to the hyperlipidemia per se rather than the atherosclerosis. Indeed, numerous studies both in vitro and in vivo have demonstrated increased platelet sensitivity and aggregability (29-33) as well as increased thromboxane production (34-36) in association with hyperlipidemia, especially hypercholesterolemia.

Thusfar we have been concerned with the effects of atherosclerosis and hyperlipidemia on prostaglandin production, but of equal importance is the possible role of prostaglandins in the production of atherosclerosis. One of the most comprehensive theories of atherogenesis is "the response to vascular injury" hypothesis (37-42), which states that atherosclerosis is a pathologic response to vessel wall injury. In response to vascular endothelial damage, platelet adherence to exposed collagen and subsequent smooth muscle cell migration and proliferation occur. As a result, the vascular endothelium is regenerated. However, repetitive vascular injury and/or chronic exposure to lipid peroxides may induce inappropriate chronic platelet adherence, activation, and thromboxane production, leading to a pathologic "repair" process, that is, one that involves the beginning of an atherosclerotic plaque. Support for the role of platelets and prostaglandins in this setting comes from the observation that aspirin (an inhibitor of platelet function and prostaglandin synthesis) as well as antiplatelet serum can inhibit the development of atherosclerosis (43, 44).

In summary, atherosclerosis and hyperlipidemia appear to exert an important influence on thromboxane and prostacyclin synthesis. In turn, prostaglandins appear to play a potentially crucial role in the response of the vascular endothelium to injury.

B. Diabetes Mellitus Accelerated disease of both the macro and microcirculation accounts for a great deal of the morbidity and premature mortality of diabetes mellitus. It is well-established that diabetes is associated with alterations in prostaglandin production and platelet function. Spontaneously diabetic rats (45), streptozotocin-induced diabetic rats (46), and diabetic humans (47-51) all have hyperreactive platelets with increased thromboxane generation in response to ADP, epinephrine, collagen, and arachidonic acid. At the same time, several studies have demonstrated reduced vascular PGI_2 synthesis in experimental and human diabetes (46, 52-57).

The link between these observations of altered prostaglandin synthesis and platelet function in diabetes with the development of diabetic vascular disease and atherosclerosis has not been established. Therapeutic attempts to reestablish the altered thromboxane:prostacyclin "balance" in diabetic patients and to avert diabetic vascular complications are underway in clinical trials. It is hoped that the pharmacologic blockade of TxA_2 production, inhibition of platelet function, and stimulation of PGI_2-like activity will forestall the vascular complications of this disease.

C. Smoking Cigarette smoking has been strongly linked with the development of coronary atherosclerosis (58, 59). In addition, epidemiologic data suggest that cigarette smoking can trigger acute coronary events both in patients with underlying atherosclerotic coronary artery disease (60, 61) as well as in those without such underlying disease (62). The mechanism(s) by which smoking exerts its effects are unknown, but some evidence suggests that a thromboxane:prostacyclin imbalance is important. First, cigarette smoking has been shown to increase platelet activity (63, 64) and proaggregatory prostaglandin production (65). Second, smoking potentiates the effects of hyperlipidemia on platelet aggregability (66). This observation is of special interest, because it supports the clinical impression that smoking alone is a relatively weak risk factor, but in combination with hyperlipidemia it is more substantial (67). Third, in vitro studies have shown that nicotine unfavorably alters the thromboxane:prostacyclin balance by reducing PGI_2-like activity (68, 69). This adverse effect of nicotine is more pronounced in vascular tissue of patients who smoke than in tissue from non-smoking controls (70). In short, smoking, and nicotine itself, alter prostaglandin production and platelet function in ways that are potentially deleterious.

D. Hypertension Because of the potent vasoactive influence of both thromboxane and prostacyclin, it is not surprising that a thromboxane:prostacyclin imbalance has been suggested as the primary pathophysiologic alteration in at least some patients with essential hypertension. In support of this hypothesis, Grose, et al (71) have recently shown that patients with essential hypertension have a reduced urinary concentration

of 6-Keto-PGF_{1a} (the major metabolite of prostacyclin) and a normal concentration of thromboxane B_2, whereas individuals without hypertension have a normal urinary concentration of both substances. Thus, this preliminary report demonstrates a thromboxane:prostacyclin imbalance in patients with essential hypertension.

Although alterations in the thromboxane:prostacyclin ratio may be of primary importance in patients with essential hypertension, they may also occur in response to chronic elevations or depressions of other vasoactive substances. For example, vascular tissue from the spontaneously hypertensive rat has been shown to have an enhanced ability to produce PGI_2 (72), presumably a reactive response of the vascular tissue to chronic elevations of circulating catecholamines (73). Interestingly, it appears that this enhanced capacity to produce PGI_2 is protective against vascular catastrophies, since the frequency of cerebrovascular accidents increases markedly when this enhanced capacity is lost (74). Thus, PGI_2 may serve to attenuate the hypertensive, vasoconstrictive, and proaggregatory effects of hypertensive stimuli, such as catecholamines.

Certain pharmacologic inhibitors of prostaglandin production may exert important effects on hypertension and its therapy. First, indomethacin, a cyclooxygenase inhibitor which blocks the synthesis of all prostaglandins and thromboxanes, has been shown to elevate blood pressure when given alone (75) and to block the anti-hypertensive actions of furosemide (75) and propranolol (76). Second, sulindac, another cyclooxygenase inhibitor, has been reported to induce a hypertensive response in an occasional patient (77). Third, it is known that certain endogenous prostaglandins cause vasodilatation, natriuresis, diuresis, or renin release (78, 79). In short, the pharmacologic control of hypertension may depend, at least in part, on the effect of the various anti-hypertensive agents on thromboxane and prostaglandin synthesis and release.

In summary, it appears that (a) alterations in thromboxane:prostacyclin balance exist in at least some patients with essential hypertension; (b) some of these alterations may be protective, whereas others are clearly detrimental; and (c) pharmacologic interventions directed at selectively enhancing the protective prostaglandins and/or reducing the harmful ones may prove useful in the future.

E/F. Sex Hormones/Age It is well-established that men have a higher cardiovascular morbidity and mortality than women (80), but this difference appears to be influenced both by age and by the presence of circulating sex hormones. First, the marked difference in the frequency of cardiovascular events between young men and women is reduced in the post-menopausal period (81). Second, cardiovascular risk is increased in men and post-menopausal women treated with estrogenic hormones (82, 83) as well as in young women on oral contraceptives (84-86). Third, physical signs of "feminization" in men are associated with an increased

risk of myocardial infarction (87). In such patients, serum estradiol and estrone concentrations are elevated, and serum testosterone or dihydrotestosterone levels are normal when compared to controls (88). Fourth, the hypercoagulable state noted in patients with Takayasu's disease correlates with continuously elevated estrogen production (89). Thus, from epidemiological and clinical studies, it appears that estrogens represent a cardiovascular "risk factor."

Although attempts have been made to link this hormonal-dependent risk factor to various alterations in lipoprotein concentrations, there is not a good relationship (90). However, certain hormonal-induced alterations in prostaglandin production and platelet aggregability correlate with some of the clinical observations cited above. Many studies suggest that estrogens exert an adverse effect on both cardiovascular risk and thromboxane:prostacyclin equilibrium. First, platelet aggregability is reportedly increased in young and middle-aged women in comparison to age-matched men (91, 92). Second, despite conflicting reports to the contrary (93), most investigators have observed that estrogen administration to experimental animals (94) and man (95) causes increased platelet aggregability, with a concomitant reduction in the vascular production of prostacyclin-like substances (94). Third, spontaneous platelet aggregation occurs in vitro in blood from young women on oral contraceptives but not in young healthy men and women on no treatment, and this is abolished by aspirin therapy. Fourth, platelets are more reactive in the highly estrogenic follicular phase of the menstrual cycle than during the luteal phase (when both estrogen and progesterone are high). Fifth, the precipitous fall in plasma progesterone levels with menopause (96) correlates temporally with an increase in platelet aggregability. Lastly, the gradual increase in cardiovascular risk in men with advancing age is related temporally with increased conversion of androstenedione to estrone (97, 98).

In short, the effect of sex and, to some extent, age on the risk of cardiovascular events may be related to alterations in prostaglandin production and release. Further studies are needed to establish more clearly the exact role of these substances. The sex-specific beneficial influence of aspirin in the prevention of threatened stroke (99) and venous thromboembolism (100) certainly suggests that prostaglandin production and release are affected substantially by these factors.

G. Heredity Those individuals with a strong family history of premature coronary artery disease are at increased risk for the early development of such disease, and some studies have demonstrated an increased incidence of platelet functional abnormalities in asymptomatic individuals in whom there is a strong family history of coronary artery disease (101). The recent demonstration of 3 generations of a single family with thromboxane synthetase deficiency (20) suggests that the activity of this and other important regulator enzymes may be genetically controlled. Even if the resultant alterations in thromboxane and prostacyclin production are relatively subtle, they may, nonetheless,

account for some of the familial propensities for the development of premature coronary artery disease.

That a genetic disease of prostaglandin imbalance causes a clinically-important syndrome is supported by Bartter's syndrome (102-104). Admittedly, it is uncertain whether Bartter's is a primary or secondary alteration in prostaglandin production. Despite this, it is interesting that pharmacologic strategies designed to normalize the prostaglandin alteration have been successful in reversing the insensitivity to angiotensin, the high plasma renin and aldosterone levels, and the resultant hypokalemia.

H. Stress Catecholamines, important humoral mediators of stress, are used routinely in platelet research because of their proaggregatory effects. Although the link between emotional stress and platelet functional abnormalities is unproven in vivo, studies performed in a group of healthy volunteers (medical house officers) have shown that such stress causes marked platelet functional alterations (105, 106). Apart from their influence on platelets, catecholamines also directly stimulate prostaglandin synthesis. It seems plausible, then, that some cardiovascular events are mediated by catecholamine-induced activation of thromboxane and prostacyclin production. In fact, catecholamine-mediated platelet aggregation and thromboxane synthesis can be blocked by cyclooxygenase and thromboxane synthetase inhibitors (107).

I. Diet The interrelationships of hyperlipidemia, atherosclerosis, and prostaglandins have been discussed. Therefore, this consideration of diet as a cardiovascular risk factor will focus on the unique properties of several essential fatty acids. Greenland eskimos have a reduced incidence of vascular disease as well as a prolonged bleeding time. In addition, they have a high dietary intake of fish, which contains large quantities of the fatty acid, eicosapentaenoic acid (EPA)(108). In contrast to eicosatetraenoic acid (arachidonic acid), EPA is metabolized to prostaglandins and thromboxanes of the "3" series (3 double bonds), PGI_3 and TxA_3 (109) (Figure 3, page 15). Although PGI_3 is similar in activity to PGI_2, TxA_3 is physiologically inactive (110) and, in fact, may actually block the platelet TxA_2 receptors, thus inhibiting pro-aggregatory stimuli (111). Therefore, by serving as the parent compound of different prostaglandin end-products, EPA promotes vascular dilatation and inhibits platelet aggregation. As a result, these eskimos have a reduced cardiovascular risk (112).

The feeding of a diet rich in mackerel (113) or cod liver oil (114) (both high in EPA) to healthy Caucasian men causes similar alterations of prostaglandin production, with hemostatic and prostaglandin changes similar to those found in the Greenland eskimos. Thus, it appears that dietary manipulation can effectively alter prostaglandin metabolism. Whether this, in itself, can alter cardiovascular risk is an unproven hypothesis with profound implications for Western society.

FIGURE 3

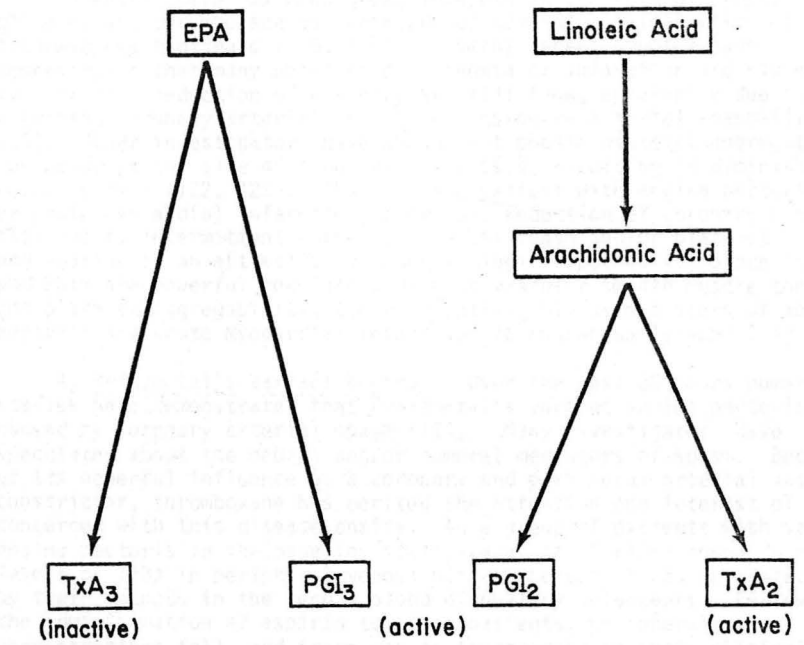

Figure 3 A schematic representation of how dietary manipulation may lead to a relative abundance of prostacyclin. The normal American diet contains an abundance of arachidonic acid precursors, such as linoleic acid. Arachidonic acid is metabolized to PGI_2 and TxA_2, both active compounds. In contrast, makerel and cod contain a large quantity of eicosapentaenoic acid (EPA), which is metabolized to prostaglandins and thromboxanes of the "3" series. Although PGI_3 is similar in activity to PGI_2, TxA_3 is physiologically inactive. Thus, by serving as the parent compound of different prostaglandin end-products, EPA promotes vascular dilatation and inhibits platelet aggregation. As a result, the Greenland eskimos who eat a large amount of mackerel have a reduced incidence of vascular disease as well as a prolonged bleeding time.

IV. PROSTAGLANDINS AND ISCHEMIC HEART DISEASE

Recently there has been great interest in the role of prostaglandins and thromboxane as mediators of several clinical forms of ischemic heart disease (115, 116). Several recent studies have demonstrated that many episodes of ischemia or infarction are caused by a primary reduction of coronary arterial flow, apparently due to augmented coronary arterial tone (i.e., coronary arterial spasm)(117-121). Other investigators have shown that phasic platelet aggregation may occur at the site of a coronary stenosis, resulting in diminished coronary flow (122, 123). Thus, in the patient with angina pectoris or acute myocardial infarction, a primary reduction of coronary blood flow due to intermittent coronary arterial spasm and/or platelet aggregation is an attractive pathophysiologic hypothesis. Since TxA_2 and PGI_2 are powerful modulators of both vascular smooth muscle tone and platelet aggregability, their potential role as mediators of angina pectoris and acute myocardial infarction is conceptually appealing.

A. Prinzmetal's Variant Angina Over the past 20 years numerous studies have demonstrated that Prinzmetal's variant angina pectoris is caused by coronary arterial spasm (124). Many investigators have speculated about the neural and/or humoral mediators of spasm. Because of its powerful influence as a coronary and peripheral arterial vasoconstrictor, thromboxane has merited the attention and interest of those concerned with this disease entity. In a group of patients with variant angina pectoris in the baseline state, Lewy, et al (125) found detectable levels of TxB_2 in peripheral venous blood, whereas it was not detectable by their methods in the venous blood of healthy volunteers. Following the administration of aspirin to these patients, peripheral venous TxB_2 concentrations fell, and there was an improvement in their clinical syndrome (126). From these studies, Lewy and his associates concluded that "thromboxane generation appears to be a pathogenetic factor in Prinzmetal's angina."

Subsequent studies have further clarified the course of thromboxane elevations in the blood of patients with variant angina pectoris, but they have cast serious doubt on the pathogenetic role of thromboxane alone in this syndrome. In 8 patients with variant angina, Lewy, et al (127) found that peripheral venous thromboxane concentrations in the baseline state (not in close proximity to chest discomfort) were barely measurable and that they were unchanged during an episode of chest pain. In contrast, several minutes after the resolution of chest discomfort, TxB_2 levels in peripheral venous blood were increased several-fold. Utilizing samples of blood obtained directly from the coronary sinus, Robertson, et al (128) confirmed Lewy's impression that thromboxane elevations do not precede the onset of an episode of variant angina. These investigators demonstrated that coronary sinus TxB_2 concentrations

drawn within 2 minutes before or 30 seconds after the onset of ST segment elevation were not abnormally elevated. However, after ST segment elevation was present for 2-3 minutes, coronary sinus TxB_2 levels rose precipitously and were highest several minutes after chest pain and ST segment elevation resolved. In short, the observations of both Lewy, et al (127) and Robertson, et al (128) suggest that elevations of TxB_2 in patients with Prinzmetal's variant angina pectoris are the result, not the cause, of this clinical syndrome (Figure 4, page 18).

That thromboxane is not an important causal factor in the generation of coronary arterial spasm is supported by the studies of both Robertson, et al (129, 130) and Chierchia, et al (131). Both groups demonstrated that certain cyclooxygenase inhibitors, such as aspirin and indomethacin, effectively blunt the increase in coronary sinus TxB_2 concentrations that occurs immediately following an episode of variant angina. Despite this, neither agent has any influence on the frequency, severity, or duration of chest discomfort. In fact, Robertson, et al (129) have studied low-dose aspirin and indomethacin in a double-blind, crossover fashion and have found that neither of these pharmacologic agents affects the clinical syndrome in any way.

Therefore, thromboxane alone does not appear to be the humoral cause of coronary arterial spasm. Nevertheless, it may play some pathogenetic role in combination with other humoral or neural events. For example, if the hypothesis of a thromboxane:prostacyclin balance in the control of vascular tone and platelet function is valid, the studies cited above may have looked at half the puzzle. It is possible that coronary arterial spasm is induced because of a transient alteratic in the coronary thromboxane:prostacyclin ratio. The answers to these questions are not presently available.

B. Stable (Exertional) Angina Although a number of studies have attempted to examine the possible contribution of prostaglandins in patients with stable, exertional angina pectoris, the results have been inconsistent and confusing. In a group of individuals with severe atherosclerotic coronary artery disease and stable angina, Berger, et al (132) demonstrated a distinct increase in the coronary sinus concentration of PGF during pacing-induced angina. The significance of this observation is unclear. Although prostaglandins of the "F" series have little effect on coronary or systemic hemodynamics in animals or man (133, 134), their administration in experimental myocardial infarction results in improved survival and decreased release of creatine kinase and lysosomal enzymes following coronary occlusion (135).

More recently, Lewy et al (136) have shown that rapid atrial pacing sufficient to induce angina is accompanied by a modest increase in coronary sinus TxB_2 levels; however, during the minutes after pacing has been discontinued and angina has resolved, these TxB_2 concentrations rise precipitously, reaching a maximum value 5-10 minutes post-pacing. From these studies, therefore, there appears to be a good relationship between pacing-induced myocardial ischemia and the release into the coronary circulation of several prostaglandins and thromboxane. Because

FIGURE 4

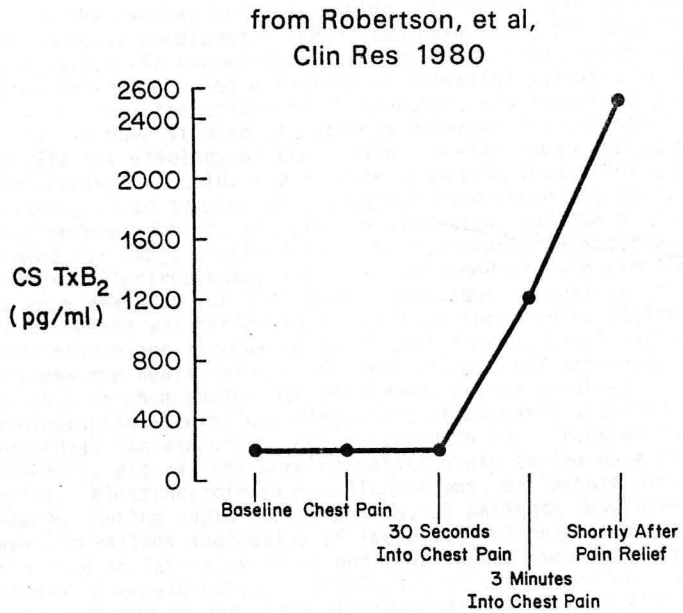

Figure 4 Coronary sinus thromboxane B_2 (CS TxB_2) concentrations, in picograms/milliliter (pg/ml), in 3 patients with variant angina pectoris in the baseline state, early during chest pain, 30 seconds into chest pain, 3 minutes into chest pain, and shortly after pain relief. Note that CS TxB_2 levels are not elevated either before or early into chest pain, but they begin to rise 3 minutes into chest pain and reach a maximum value immediately after pain relief. The authors conclude that thromboxane alone is not the cause of Prinzmetal's variant angina. From Reference #128.

of its powerful vasoactive properties, thromboxane release at the time of ischemia has received considerable attention. However, these studies do not elucidate the sequence in which myocardial ischemia and thromboxane production and release occur.

In the Cardiac Catheterization Laboratory here at Parkland, we have recently completed a large study examining the transcardiac thromboxane and prostacyclin concentrations in patients with stable, exertional angina pectoris during a variety of stressful stimuli, including rapid atrial pacing, the cold pressor maneuver, and isometric exercise (137). In all, we have studied 54 patients referred for catheterization to evaluate the etiology of chest pain: 24 with rapid atrial pacing, 20 with exposure to cold, and 10 with isometric (handgrip) exercise. In all patients, blood samples were obtained from ascending aorta and coronary sinus before, during, and after provocation, and the blood samples were assayed for TxB_2, 6-keto-$PGF_{1\alpha}$ (the measurable metabolite of PGI_2), and lactate. Electrocardiograms were performed at each sampling interval and were analyzed for ST-T wave alterations suggestive of ischemia. Rapid atrial pacing was performed in 3 minute increments, beginning at 100 beats/minute and increasing by 15 beats/minute until angina occurred or a maximum heart rate of 160 beats/minute was achieved. In the 24 patients in whom pacing was performed, angina occurred in 17, electrocardiographic alterations diagnostic of ischemia occurred in 12, and myocardial lactate production occurred in 13. Transcardiac TxB_2 and 6-keto-$PGF_{1\alpha}$ alterations bore no relationship to the development of angina, electrocardiographic alterations, or lactate production. For example, during rapid atrial pacing, 12 patients developed obvious ST-T wave alterations indicative of ischemia, yet only 2 developed substantial transcardiac TxB_2 elevations and none showed important transcardiac changes in 6-keto-$PGF_{1\alpha}$. Similarly, of the 13 patients who produced lactate during pacing, only one demonstrated an elevation in transcardiac TxB_2 concentration. Provocation with other methods--cold pressor (immersion of the patient's hand in ice for 3 minutes) and isometric exercise (handgrip to 30% of maximum force development for 3 minutes)--did not produce angina, electrocardiographic alterations, or lactate production, and transcardiac TxB_2 and 6-keto-$PGF_{1\alpha}$ levels did not change. In short, our studies in patients with stable angina have failed to demonstrate a consistent relationship between provoked myocardial ischemia--as confirmed by the appearance of angina, electrocardiographic alterations, and myocardial lactate production--and alterations in transcardiac thromboxane and prostacyclin concentrations.

Although coronary arterial spasm or transient platelet "plugging" may be operative in some patients with angina of effort, most individuals with this disease entity develop myocardial ischemia because of excessive myocardial oxygen demand in the setting of limited oxygen supply. A primary reduction in blood supply to a portion of myocardium (induced by an increase in coronary arterial tone and/or a transient platelet "plug") probably is <u>not</u> of pathophysiologic importance in most patients with angina of effort. <u>Rather</u>, these patients develop angina simply because of a transient increase in oxygen demand.

C. Unstable Angina Unstable angina pectoris is a clinical syndrome intermediate between chronic stable angina and acute myocardial infarction. Over the past several years there has been intense interest in the identification of the factor(s) responsible for the conversion of stable to unstable angina pectoris. Attention has focused on the possible role of thromboxane and prostacyclin in this clinical syndrome. Indeed, recent studies from this institution and elsewhere suggest that these substances may contribute to the production of instability in the patient with previously stable angina.

First, patients with known ischemic heart disease have been demonstrated to have hyperreactive platelets, which have both augmented thromboxane production and sensitivity (138). Mehta, et al (139, 140) have confirmed these observations, demonstrating in vitro that platelets from patients with angina pectoris and severe underlying coronary artery disease exhibit reduced sensitivity to prostacyclin when compared to those from healthy volunteers. These abnormal platelet sensitivities may be important potential mechanisms in the pathogenesis of myocardial ischemia. Second, recent studies from our laboratory (141, 142) have shown that patients with unstable angina pectoris and continuing clinical instability have distinctly elevated transcardiac levels of thromboxane when compared to patients without coronary artery disease and those with stable angina pectoris..

In 60 patients referred for catheterization for the evaluation of various cardiac disorders, ascending aortic and coronary sinus blood samples were obtained prior to heparinization and the introduction of iodinated contrast material. On the basis of the history, non-invasive evaluation, and results of catheterization, and without knowledge of the prostaglandin, thromboxane, or lactate results, each patient was assigned to one of 5 groups. Group A (n=6) had non-ischemic heart disease, including a variety of congenital and acquired non-coronary cardiac lesions. Specifically, these patients had patent ductus arteriosus, atrial septal defect, mitral stenosis, mitral regurgitation, idiopathic (non-ischemic) cardiomyopathy, and cor pulmonale secondary to primary pulmonary hypertension. Group B (n=14) was composed of patients with a syndrome of chest pain without objective evidence of cardiac disease by resting electrocardiogram, ambulatory two-channel electrocardiographic monitoring, exercise tolerance testing with simultaneous radionuclide equilibrium gated blood pool scintigraphy, and cardiac catheterization, including selective coronary arteriography with ergonovine provocation. In Group C (n=18), each patient had ischemic heart disease with the most recent episode of chest pain more than 96 hours prior to study. In Group D (n=15), each patient had ischemic heart disease, and the most recent chest pain occurred 24-96 hours prior to study. Finally, Group E(n=7) was composed of patients with ischemic heart disease and chest pain within 24 hours of study. One patient who sustained an acute subendocardial myocardial infarction 12 hours following the study (confirmed by serum enzymes, including CK-B, and serial technetium-99m-stannous pyrophosphate myocardial scintigraphy) was included in Group E.

For each patient, the coronary sinus (CS) and ascending aortic (Ao) concentrations of TxB_2 and 6-keto-$PGF_{1\alpha}$ were expressed as the ratio of CS/Ao, since we were primarily interested in the transcardiac changes in these substances. For TxB_2, significant inter-group differences were observed among the 5 groups (Figure 5A, page 22). Group A (valvular and congenital non-ischemic heart disease) had a TxB_2 CS/Ao ratio of 1.2 ± 0.6 (median 1.2, range 0.2 - 1.9); Group B (chest pain syndrome without ischemic heart disease) had a TxB_2 CS/Ao ratio of 1.2 ± 0.4 (median 1.2, range 0.4 - 2.2); and Group C (ischemic heart disease without chest pain for at least 96 hours) had a TxB_2 CS/Ao ratio of 1.3 ± 0.6 (median 1.2, range 0.5 - 3.0). Although the overall mean TxB_2 CS/Ao ratio for Group D (ischemic heart disease with chest pain 24-96 hours prior to study) was 6.3 ± 12.5 (median 1.5, range 0.5 - 46.6), this group demonstrated a distinct bimodal distribution: 12 patients had low TxB_2 CS/Ao ratios (mean 1.3 ± 0.4, range 0.5 - 2.1), whereas 3 patients had markedly elevated values (mean 26.2 ± 18.5, range 10.5 - 46.6). The patients in Group D with low and high TxB_2 CS/Ao ratios could not be differentiated on the basis of clinical criteria. Group E (ischemic heart disease and chest pain within 24 hours of study) had a TxB_2 CS/Ao ratio of 5.8 ± 2.8 (median 4.5, range 3.5 - 9.9), significantly higher ($p < 0.05$) than Groups A, B, and C.

The aortic concentrations of TxB_2 allowed no distinction among Groups A-E, nor did the CS TxB_2 concentrations alone or the absolute changes in TxB_2 across the coronary bed. Similarly, the CS/Ao ratios of 6-keto-$PGF_{1\alpha}$ (a stable degradation product of PGI_2) showed no significant inter-group differences (Figure 5B, page 22). Finally, the CS/Ao ratio of TxB_2 divided by the CS/Ao ratio of 6-keto-$PGF_{1\alpha}$ was examined in an attempt to assess the contribution of a disrupted thromboxane:prostacyclin "balance" across the coronary bed; this ratio did not allow better discrimination among the 5 groups of patients than the TxB_2 CS/Ao ratio alone. All patients demonstrated myocardial lactat extraction at the time of study, and there were no inter-group differences in terms of lactate concentrations.

In short, this study demonstrates that patients with unstable angina pectoris and continued clinical instability (as manifested by continuing chest pain) have a several-fold increase of thromboxane across their coronary beds; in contrast, individuals without ischemic heart disease and those with stable angina have distinctly lower values of thromboxane across the heart. The transcardiac concentrations of 6-keto-$PGF_{1\alpha}$ demonstrate no clear relationship to the presence or degree of clinical activity of ischemic heart disease. The observation that transcardiac thromboxane is increased in close temporal proximity to an anginal epidose but is independent of transcardiac lactate production suggests that the ongoing release of thromboxane may not be a result of persistent myocardial ischemia.

The apparent lack of association between pacing-induced ischemia and transcardiac thromboxane concentrations in patients with stable,

FIGURE 5

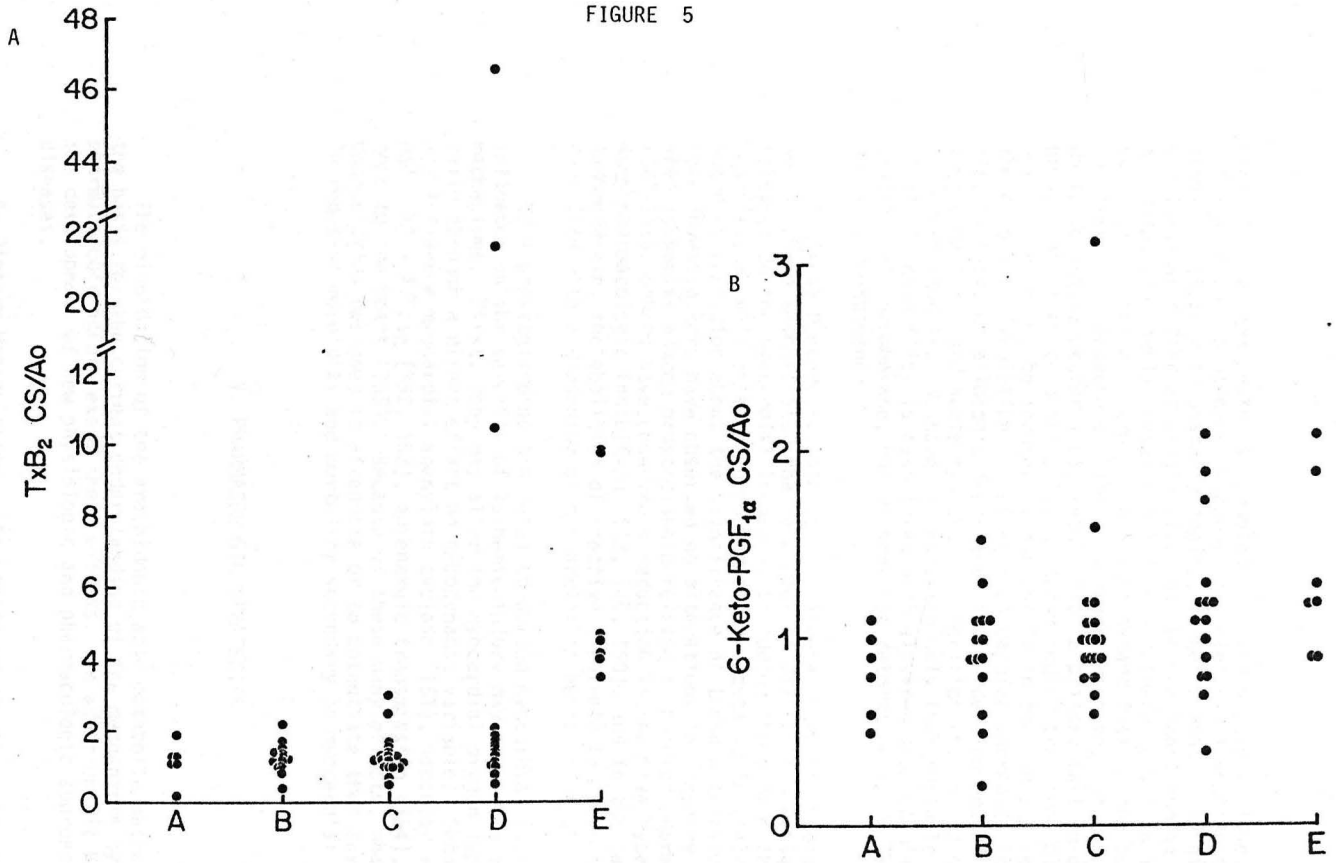

Figure 5 The TxB_2 CS/Ao ratios (left, panel A) and the 6-keto-PGF_{1a} CS/Ao ratios (right, panel B) for the 5 groups of patients. Each point represents the data from one patient. In Groups A (valvular and congenital non-ischemic heart disease), B (chest pain syndrome without ischemic heart disease), and C (ischemic heart disease without chest pain for at least 96 hours), all patients had TxB_2 CS/Ao ratios less than 3.1. Group D (ischemic heart disease with chest pain 24-96 hours prior to study) had a bimodal distribution:12 patients had low TxB_2 CS/Ao ratios, whereas 3 had very high ratios. Group E (ischemic heart disease and chest pain within 24 hours of study) had TxB_2 CS/Ao ratios ranging from 3.5 to 9.9, higher ($p < 0.05$) than Groups A, B, and C. Among the 5 groups, the 6-keto-PGF_{1a} CS/Ao ratios were not statistically different. From Reference #142.

exertional angina pectoris, coupled with the apparent close association between recent spontaneous ischemia and elevated transcardiac thromboxane levels in those with unstable angina, suggests that these disease entities may differ etiologically. On the one hand, angina of effort, as stated previously, results not from a primary reduction in oxygen supply, but from an increase in oxygen demand that cannot be accomodated satisfactorily because of limited coronary reserve. On the other hand, unstable angina pectoris may result from a primary fall in oxygen supply, brought about by coronary arterial spasm and/or transient platelet "plugging," both of which may be induced by and result in thromboxane release into the coronary circulation. Whether transcardiac increases in thromboxane are, in fact, of etiologic importance in the development of unstable angina pectoris and acute myocardial infarction remains to be defined in future studies. We have demonstrated only that unstable angina with clinical instability is associated with elevated transcardiac concentrations of thromboxane, but we have not determined which is cause and which is consequence.

D. Acute Myocardial Infarction Several studies in experimental animals have demonstrated that a number of different prostaglandins are released during myocardial ischemia, including those from the A, E, and F series, as well as both thromboxane and prostacyclin (143-146). However, there is confusion about the significance of these alterations. Although some investigators have observed no alterations in coronary blood flow when ischemia-induced prostaglandin release is blocked pharmacologically (147-149), others have observed a reduction in reactive hyperemia during such pharmacologic inhibition (143, 145, 150), and in the case of indomethacin, the abolition of reactive hyperemia has been shown to correlate with a worsening of myocardial ischemic injury.

Once prostaglandins are released during myocardial ischemia, their influence on the severity of ischemic injury may occur via several mechanisms. First, they may alter the myocardial oxygen supply:demand ratio through a direct effect on hemodynamic variables. Second, they may influence myocardial adenylate cyclase (151), vascular and myocardial Na^+ - K^+ - ATPase (152, 153), adrenergic transmission (154), and vagal tone to the heart (155). Because of these many effects, pharmacologic manipulation designed to attenuate or to potentiate them may be beneficial in reducing morbidity and mortality secondary to myocardial infarction.

V. PHARMACOLOGIC STRATEGIES

The elucidation of the arachidonic acid metabolic pathway forms the basis for the current understanding of the mechanisms by which many pharmacologic agents exert their effects. In addition, it has prompted the development of new physiologic and pharmacologic approaches to many diseases.

A. Dietary Manipulation As stated previously, eicosatetraenoic acid (arachidonic acid) is converted to TxA_2 and PGI_2, whereas

eicosapentaenoic acid (EPA) is converted to TxA_3 and PGI_3. TxA_3 is physiologically inactive, whereas PGI_3 has the same effects as PGI_2 (Figure 3, page 15). Thus, in comparison to arachidonic acid, EPA is the dietary precursor of an antithrombogenic agent.

Meat and vegetable oils are the principal dietary sources of linoleic acid, the precursor of arachidonic acid. In contrast, EPA is highly concentrated in cold water fish. Those individuals with a high dietary intake of arachidonic acid precursors have an incidence of vascular and ischemic heart disease that is elevated in comparison to those whose diet is high in EPA precursors. Therefore, an increase in the EPA:Arachidonic Acid ratio may provide a potential prophylaxis against vascular complications. It is unlikely, however, that Americans will find a diet high in mackerel and cod-liver oil palatable or tolerable.

Fortunately, there may be alternatives. Another fatty acid precursor, dihomo-gamma-linoleic acid (DLL), an eicosatrienoic acid, is metabolized to thromboxane and prostaglandins of the "1" series, that is, with one (rather than 2) double bond. Like EPA, dihomo-linoleic acid inhibits in vitro aggregation (156) and is anti-aggregatory when administered orally to man (157, 158). In one study of 7 patients, a relatively small amount of DLL supplementation effectively altered platelet reactivity despite continued normal dietary fat intake (108). Therefore, an increase in the dietary DLL:Arachidonic Acid ratio may offer an alternative whereby ischemic vascular events can be avoided.

Recently, Needleman, et al (159) have suggested that dietary EPA should be combined with a specific thromboxane synthetase inhibitor, thus almost completely abolishing thromboxane synthesis. If dietary linoleic acid is truly a cardiovascular "risk factor," this approach may represent a substantial contribution to the prevention and treatment of ischemic vascular disease.

B. Inhibition of Phospholipase Phospholipase is the enzyme which liberates arachidonic acid from the phospholipid storage pool. Therefore, agents which inhibit the formation, activiation, or action of this enzyme prevent the entry of arachidonic acid into the prostaglandin production pathway. By interfering with the synthesis of phospholipase, glucocorticosteroids block both the cyclooxygenase and the lipoxygenase pathways of arachidonic acid metabolism (160, 161) (Figure 6, page 25). Since this inhibitory effect is not seen in platelets (162) (because they are not capable of protein biosynthesis), glucocorticosteriods interfere with PGI_2 production but not thromboxane generation. The result is one of increased vascular tone and hypercoagulability (163).

The effects of blocking the lipoxygenase pathway are not fully understood, thus accounting for the unpredictable effects of glucocorticoids in various clinical situations. For example, the anti-inflammatory effect of steroids, which probably results from an inhibition of both

FIGURE 6

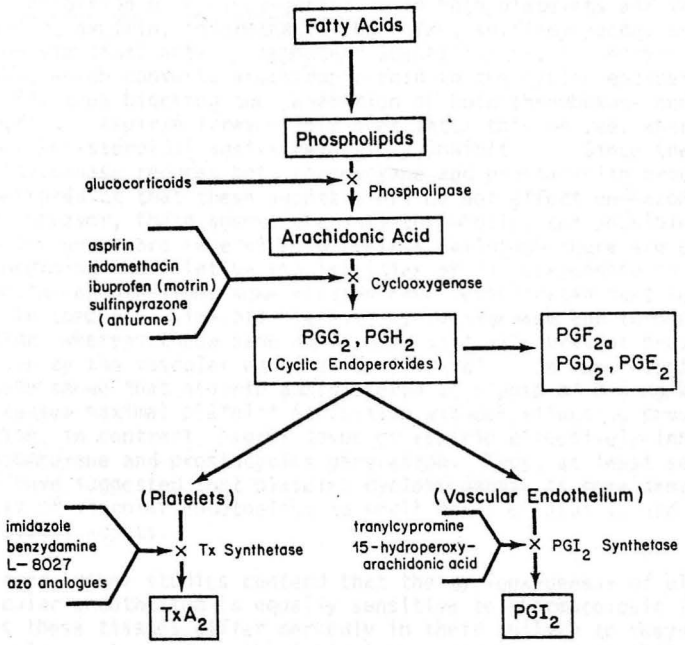

Figure 6 The pathway by which arachidonic acid is formed and then metabolized to the various prostaglandins and the pharmacologic agents which block this pathway. Glucocorticoids interfere with the phospholipases, those enzymes which cleave the phospholipids of cellular membranes to form arachidonic acid. A number of compounds inhibit cyclooxygenase activity, including aspirin, indomethacin, ibuprofen (motrin), and sulfinpyrazone (anturane); therefore, these agents prevent the eventual synthesis of both thromboxane and prostacyclin. In vascular endothelium, tranylcypromine and 15-hydroperoxy-arachidonic acid block PGI_2 synthetase activity. Several compounds are specific Tx synthetase inhibitors, including imidazole, benzydamine, L-8027, and the azo analogues. There is presently a great deal of interest in the development of a clinically usable TxA_2 synthetase inhibitor.

the cyclooxygenase and the lipoxygenase pathway, may, in certain conditions, play a greater role than their vasoconstrictive and pro-aggregatory effects. In general, pharmacologic strategies designed to inhibit arachidonic acid release from phospholipids lack specificity and, as a result, are often counterproductive.

C. Inhibition of Cyclooxygenase In both platelets and vascular endothelium, aspirin, indomethacin, ibuprofen, sulfinpyrazone, and the other non-steroidal anti-inflammatory agents inhibit the enzyme cyclo-oxygenase, which converts arachidonic acid to the cyclic endoperoxides (Figure 6), thus blocking the generation of both thromboxane and prostacyclin. Aspirin irreversibly acetylates this enzyme, whereas the other non-steroidal agents reversibly inhibit it. Since the inhibition of cyclooxygenase reduces both thromboxane and prostacyclin production, one might predict that these agents exert no net effect on thrombosis. In fact, however, these agents are anti-thrombotic, the possible reasons for which are several fold. First, although there are conflicting data concerning the relative sensitivities of cyclooxygenase in platelets and vascular endothelium, some studies have demonstrated that low doses of aspirin completely inhibit platelet cyclooxygenase and thromboxane generation, whereas these same doses only partially prevent prostacyclin production by the vascular endothelium (164-167). In man, Masotti, et al (168) have shown that aspirin administered at a dose of 3.5 mg/kg every 3 days causes maximal platelet inhibition without affecting prostacyclin production; in contrast, higher doses of aspirin effectively inhibit both thromboxane and prostacyclin generation. Thus, at least some studies have suggested that platelet cyclooxygenase is more sensitive than that of vascular endothelium to small doses of aspirin and associated pharmacologic agents.

Second, other studies contend that the cyclooxygenase of platelets and vascular endothelium is equally sensitive to pharmacologic inhibition but that these tissues differ markedly in their ability to resynthesize their cyclooxygenase after the inhibitory agent is removed (169-172). Platelets that are exposed even to very low concentrations of aspirin permanently lose cyclooxygenase activity (173). Thus, the generation of thromboxane is blocked completely until new platelets with normal cyclo-oxygenase activity are produced. In contrast, vascular endothelial cells which are exposed to aspirin lose their ability to synthesize prostacyclin for only a matter of hours. As aspirin is removed from the environs of these cells, they rapidly resynthesize new cyclooxygenase. Within several hours, enzyme activity can be measured, and it has recovered completely within 36 hours after exposure to aspirin. The ability of vascular endothelial cells to regenerate cyclooxygenase activity--and the inability of platelets to do likewise--results from the fact that vascular endothelial cells have nuclei and, therefore, can synthesize protein, whereas platelets are anucleate and cannot (174).

Despite these data, the question of "low-dose" versus "high-dose" aspirin in the prevention of thrombosis remains unclear. Clinical trials designed to assess the ability of aspirin to reduce the frequency of important cardiac events have shown that both low (175, 176) and high-dose (177-181) aspirin exerts a salutary effect, implying that a maintenance of PGI_2 production may not be of great importance in the prevention of thrombosis (182). Indeed, our own studies suggest that the role of PGI_2 in ischemic heart disease may be limited (142). Specifically, we have not observed substantial alterations in trans-cardiac 6-keto-$PGF_{1\alpha}$ in patients with ischemic heart disease under any circumstances, including stable and unstable angina or following any of several provocative maneuvers (137). It is conceivable that the vasoconstrictive and proaggregatory prostaglandins predominate in the ischemic myocardium; as a result, the benefit of inhibiting these substances may outweigh the potentially harmful effects of additional PGI_2 inhibition. Alternatively, diseased coronary arteries may be unable to generate increased amounts of PGI_2, and, therefore, there may be no deleterious effect of its blockade.

Similar to the many unanswered questions concerning aspirin in patients with ischemic heart disease, the other inhibitors of cyclo-oxygenase exert varied effects on ischemia. For instance, indomethacin has been shown to increase experimental infarct size in the dog (183, 184), whereas ibuprofen (185-188) and flurbiprofen (189) do the opposite. Whether similar discrepancies exist in man is unknown. The Upjohn Pharmaceutical Company is currently funding a major multi-center trial (coordinated by the Harvard Medical School) to examine the efficacy of ibuprofen in acute myocardial infarction. Until the results of this and other trials are available, indomethacin should be avoided in patients with myocardial infarction.

D. Inhibition of Thromboxane Synthetase Several pharmacologic agents have appeared promising because of their ability to block selectively the enzyme thromboxane synthetase (Figure 6), including imidazole, pinane TxA_2, benzydamine, L-8027, and a group of compounds known as the azo analogues. Both imidazole (190) and a substance called pinane thromboxane A_2 (191) have been shown to block increases in thromboxane following coronary occlusion, with a resultant reduction in infarct size. At the present time, however, none of these agents is clinically applicable. There is intense interest in the development of a clinically-useful thromboxane synthetase inhibitor.

E. Inhibition of Phosphodiesterase Platelet aggregability is controlled in large part by the concentration of cyclic AMP, which, in turn, is determined by the enzyme adenylate cyclase. TxA_2 exerts its proaggregatory influence by reducing platelet cAMP, whereas PGI_2 does the opposite by increasing cAMP. Once formed, cAMPis degraded to 5'AMP by the enzyme phosphodiesterase. Thus, the pharmacologic blockade of phosphodiesterase leads to an increase of cAMP, with a resultant inhibition of platelet aggregation (Figure 7, page 28). Dipyridamole (persantin)

FIGURE 7

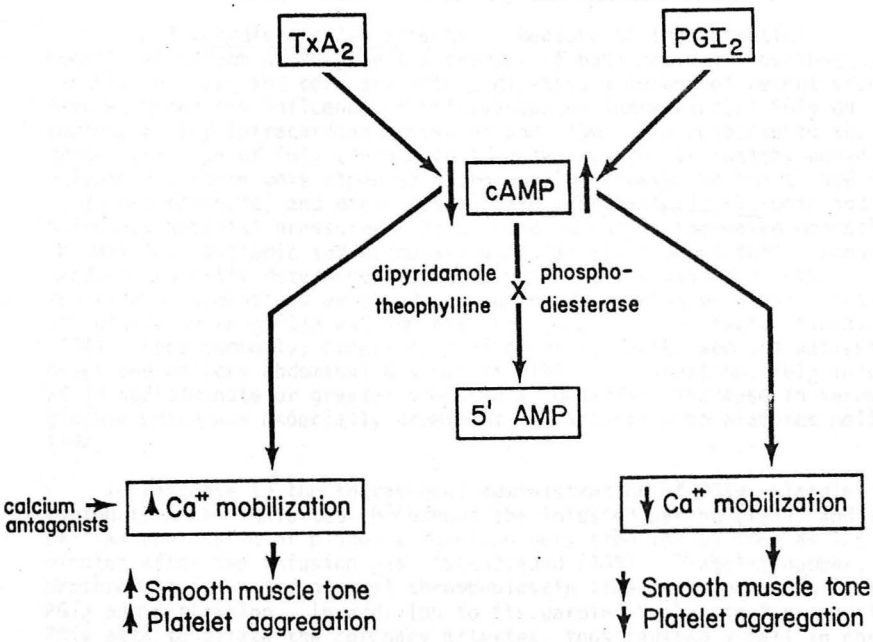

Figure 7 A schematic representation of the effects of TxA_2 and PGI_2 on platelet cAMP and its subsequent metabolism. TxA_2 reduces platelet cAMP accumulation, thus increasing calcium mobilization, which leads to an increase in smooth muscle tone and platelet aggregation. This mobilization of calcium in both arterial smooth muscle and platelets is inhibited by the calcium antagonists (verapamil, nifedipine, diltiazem, prenylamine, and perhexiline). In contrast, PGI_2 increases platelet cAMP, leading to diminished calcium mobilization, smooth muscle tone, and platelet aggregation. cAMP is metabolized by the enzyme phosphodiesterase to 5' AMP. Phosphodiesterase activity is inhibited by dipyridamole (persantin) and theophylline, thus causing an accumulation of cAMP, which leads to reduced calcium mobilization. Thus, dipyridamole inhibits platelet aggregation via a different mechanism from that of aspirin.

exerts its anti-aggregatory effect by blocking this enzyme, thus increasing cAMP levels. On clinical grounds, therefore, aspirin and dipyridamole are complimentary as anti-thrombotic agents, since they block the action of different enzymes.

F. Administration of Prostacyclin and Related Compounds

1. Cardiovascular Effects Because of the potential beneficial effect of PGI_2 in the therapy of both occlusive peripheral vascular disease and coronary artery disease, a number of recent studies have examined the influence of intravenous or intraarterial PGI_2 on peripheral and intracardiac pressures and flows. In response to the graded infusion of PGI_2 (from 2 to 16 ng/kg/minute) in healthy human volunteers, there were stepwise incremental increases in heart rate (by 15-20 beats/minute) and decreases in mean and diastolic systemic and pulmonary arterial pressures (192). Cardiac output increased modestly, so that both systemic and pulmonary vascular resistances fell. Echocardiographically-determined end-diastolic and end-systolic left ventricular dimensions were reduced during PGI_2 infusion (193). Most volunteers to whom PGI_2 was administered complained of facial flushing (194). Less commonly, others complained of headache, and one patient developed colicky abdominal discomfort (195). In addition, PGI_2 infused at 10 ng/kg/minute or greater produced a consistent increase in serum glucose which was especially prominent in patients with diabetes mellitus (196).

In response to the intravenous administration of PGI_2, platelet aggregation was inhibited throughout the infusion period (197), and a partial depression of platelet function persisted for as long as 105 minutes after the infusion was discontinued (195). Platelet number, prothrombin time, and partial thromboplastin time remained normal during PGI_2 administration. In addition to its cardiac "unloading" properties, PGI_2 acts to dilate the coronary arteries, thus causing a fall in coronary vascular resistance (198).

Although PGI_2 is reported to have a serum half-life of only 2-3 minutes, its hematological and clinical effects often are observed to last much longer. The explanation for this is unknown, but 3 hypotheses have been proposed. First, PGI_2 may be degraded in the liver to a metabolite with potent physiologic effects, such as 6-keto-PGE_1, a potent anti-aggregatory substance (199) which increases platelet cAMP levels (200), reduces blood pressure, and decreases renovascular resistance (201). Second, several recent reports have suggested that PGI_2's stability may be greatly improved once it is bound to serum albumin (202-204); as a result, its in vivo half-life may be longer than previously believed. Third, the prolonged beneficial clinical effect of PGI_2 may result from its interruption of a vicious cycle of platelet aggregation, thromboxane release, vasoconstriction, reduced blood flow, and further platelet aggregation.

2. Prinzmetal's Variant Angina There is only one preliminary report on the effects of PGI_2 in patients with "vasospastic" angina (205). In 5 patients with frequent rest angina, 3-hour infusions of PGI_2 (6-26 ng/kg/min) were alternated with those of placebo, and the response to therapy was assessed by continuous electrocardiographic monitoring. There was no difference between PGI_2 and placebo in the number, duration, or severity of ischemic episodes, although one patient had complete abolition of ischemic episodes with PGI_2.

3. Stable (Exertional) Angina Prostacyclin will never be used in the treatment of stable (exertional) angina because of its instability ex vivo, its extremely short duration of action in vivo, and the requirement for its parenteral administration. However, if it is effective in this disorder, interest will be sustained in search of a stable, long-acting analogue suitable for non-parenteral administration. Disappointingly, evidence to date suggests that PGI_2 is of no benefit in patients with stable angina during atrial pacing (206). This clinical observation is in concert with our data, which suggest that neither prostacyclin nor thromboxane is important in the pathophysiology of stable (exertional) angina or pacing-induced angina (137).

4. Unstable Angina Unstable angina is the one area of ischemic heart disease in which prostaglandin therapy may make a beneficial contribution. Recent studies (119-121) have suggested strongly that in many patients this clinical syndrome is due to transient primary reductions in coronary blood flow caused by either coronary arterial spasm or transient platelet "plugging." In experimental animals with coronary arterial stenoses, PGI_2 and its analogues have been shown to prevent the periodic interruption of coronary flow caused by platelet buildup (207, 208). As discussed earlier, our clinical data (141, 142) suggest a unique association between unstable angina pectoris and transcardiac thromboxane elevations, although these studies do not establish a causal role for thromboxane in this syndrome.

Presently several centers outside the United States are beginning to assess the utility of intravenous PGI_2 and PGE_1 (a prostaglandin with actions similar to PGI_2) in the treatment of patients with unstable angina pectoris. First, in a series of 35 consecutive patients admitted with a diagnosis of unstable angina, Bierenbaum and Oudhof (209) randomly assigned 18 to routine medical therapy (nitrates and beta-blockers) and 17 to an intravenous infusion of PGE_1. Of the 18 given the usual medical therapy, 13 evolved myocardial infarctions. In contrast, of the 17 treated with PGE_1, only 5 had infarctions ($p = 0.01$). The PGE_1 was administered at 0.5 - 1.0 micrograms/minute and, at this infusion rate, caused no adverse reactions. From this preliminary report, PGI_2 and PGE_1 appear promising in the therapy of unstable angina pectoris. However,

these data are confusing, in that the frequency of myocardial infarction in the patients receiving routine medical therapy was considerably higher than that reported in previous studies (209). Second, Szczeklik, et al (206) recently have demonstrated that intravenous PGI_2 leads to sustained and long-lasting improvement in patients with spontaneous angina at rest, as manifested by a reduction of both anginal episodes and nitroglycerin utilization. Further randomized and blinded studies of PGE_1 and PGI_2 in patients with unstable angina pectoris are needed.

5. Acute Myocardial Infarction In the experimental animal with coronary artery occlusion, the infusion of PGE_1 or PGI_2 reduces the severity of myocardial ischemic injury (211-213). Reports on the effects of prostaglandin infusions on ventricular arrhythmias are conflicting. PGE_2 has been shown to reduce the frequency of fatal ventricular arrhythmias after coronary artery occlusion in the rat (214) and dog (215), but PGE_1 and PGI_2 have been shown to enhance the vulnerability to fatal ventricular arrhythmias both in the nonischemic (216) and the ischemic myocardium (214, 216, 217), even in doses low enough to be without systemic hemodynamic effects (218).

6. Peripheral Vascular Disease Over the past 2 years several reports have described the use of intravenous or intraarterial PGI_2 in the therapy of severe, symptomatic atherosclerotic peripheral vascular disease and vasospastic peripheral arterial disease (Raynaud's disease). In 10 patients with atherosclerotic peripheral vascular disease, PGI_2 was infused into the femoral artery or subclavian vein at a rate of 2-10 ng/kg/minute for 72 hours. In response to the infusion, heart rate rose and systemic arterial pressure fell in a dose-dependent manner. Systemic vascular resistance was diminished markedly. During the infusion, symptoms of intermittent claudication improved considerably, and skeletal muscle blood flow (measured by a xenon-133 washout technique) was greatly improved. Subsequent to the 72 hour infusion, subjective and objective evidence of improvement was present for as long as several months. In fact, some patients had a sustained improvement in claudication for 7 months post-PGI_2 infusion. Muscle blood flow remained elevated for 4-6 weeks after infusion. In some patients, ischemic ulcers and limb necrosis regressed completely (192, 219, 220). No side effects were noted.

The mechanism(s) whereby PGI_2 is salutary in the patient with atherosclerotic peripheral vascular disease are unknown. PGI_2 has the capability to dissolve already-existing platelet thrombi. Therefore, it may actually "clear" capillaries of obstructing platelet deposits. Alternatively, some investigators have suggested that PGI_2 stimulates the growth and development of new capillaries in the ischemic areas. Whatever its mechanism(s) of action, it probably exerts its effect by altering flow at the arteriolar and/or capillary levels, since angiography performed after PGI_2 infusion is identical to that done before infusion (219), demonstrating that the larger arteries are not altered by PGI_2.

A similar beneficial effect has been reported for intravenous PGE_1 in the therapy of patients with Raynaud's disease (221). Over a 72 hour period, PGE_1, a potent vasodilator similar to PGI_2, was infused intravenously. Almost all the patients reported an immediate symptomatic improvement. As with the aforementioned patients with atherosclerotic peripheral vascular disease, many of the individuals with Raynaud's disease had continued symptomatic improvement for as long as 6 weeks after PGE_1 infusion. Specifically, 2 weeks after infusion, 21 of the 26 patients had continued overall improvement, and 6 weeks after infusion, 17 claimed continued benefit.

VI. CONCLUSIONS

There is a substantial amount of very suggestive information that prostaglandins are involved in an important way in the development of atherosclerotic coronary artery disease as well as in the manner in which such disease becomes clinically manifest. First, many studies demonstrate a strong relationship between prostaglandins and the numerous risk factors for peripheral and coronary artery disease. Thus, the mechanism(s) by which hyperlipidemia, diabetes mellitus, smoking, hypertension, sex, age, heredity, emotional stress, and diet contribute to the development and progression of atherosclerotic disease may be an imbalance between thromboxane and prostacyclin. Second, either with or without concomitant atherosclerotic coronary artery disease, recent studies show at least a temporal relationship between acute ischemic events (i.e., unstable angina pectoris) and a transient transcardiac imbalance between thromboxane and prostacyclin, and other studies demonstrate an apparent salutary effect of vasodilatory prostaglandins in patients with unstable angina. As with most other fields of scientific endeavor, however, these preliminary data only produce more unanswered questions. If prostaglandins and thromboxane eventually prove important in patients with ischemic vascular disease, attention will be directed at the physiologic and pharmacologic correction of whatever imbalance is operative, including dietary manipulation as well as the development of more specific enzyme inhibitors than are today available.

ACKNOWLEDGEMENTS

My very special thanks to Dr. Paul Hirsh (Cardiovascular Division) and Dr. Bill Campbell (Pharmacology Department) in the performance of our studies and the preparation of this Grand Rounds.

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