PHYSIOLOGICAL AND PHARMACOLOGICAL ROLES OF PROSTAGLANDINS[1]

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INTRODUCTION

In 1930, Kurzrok & Lieb first described the effects of seminal plasma on uterine smooth muscle strips (1). These initial observations were extended by the studies of Goldblatt and Euler, and in 1937 Euler coined the term "prostaglandin" to describe the active principle of seminal fluid (2–7). Although the potent smooth muscle stimulating and vasodepressor actions of these lipid extracts were described almost 40 years ago, further developments in the field awaited the isolation in pure form of the first two prostaglandins (8–11). The studies of Bergström and co-workers led to the identification and synthesis of at least 16 naturally occurring prostaglandins and a number of prostaglandin analogs. The availability of these substances in adequate quantities in crystalline form opened up many new avenues for research into the physiology, biochemistry, and pharmacology of these biologically active lipids. Comprehensive reviews by Bergström and co-workers and Weeks on the biology of the prostaglandins covered the literature through 1972 (12, 13). The remarkable discovery that "aspirin-like" drugs inhibit the biosynthesis of prostaglandins provided the biologist with an important tool for the investigation of physiological roles of these substances and their implication in the pathogenesis of a number of disease states (14, 15). Drugs that inhibit prostaglandin biosynthesis and newer aspects of the mode of action of nonsteroidal anti-inflammatory drugs have been reviewed recently (16, 17). A survey of recent reviews on the prostaglandins reveals that the effects of the prostaglandins on the pulmonary circulation have received little attention (12, 13). Hence, the main purpose of the present report is to provide for the first time a comprehensive review of the effects of prostaglandins

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on pulmonary circulation and on isolated intrapulmonary artery, vein, and bronchus. In addition, recent developments concerned with the role of prostaglandins as modulators in the autonomic nervous system are reviewed.

EFFECTS OF THE PROSTAGLANDINS ON PULMONARY CIRCULATION

The lung has been shown to contain large amounts of prostaglandins, and the biosynthetic capacity of the organ to form prostaglandins as measured by the conversion of tritiated di-homo-γ-linolenic acid to PGE_1 is comparable to the kidney medulla and higher than most organ systems except for the seminal vesicle (16, 18–22). Prostaglandin synthetase activity is high in the lung, and a variety of physiologic and pathophysiologic stimuli including hypoxia, anaphylaxis, hyperinflation, distention, and pulmonary edema release prostaglandins from this organ (16, 23–26). In addition to synthesis and release, the lung is an important organ for the metabolism of prostaglandins, and E and F type compounds are rapidly inactivated in the lung (27–29). The major pathway for metabolism in the lung is the oxidation of the 15-hydroxyl group to a ketone by the enzyme 15-hydroxyprostaglandin dehydrogenase (30–33). PGA type compounds are somewhat more resistant to pulmonary inactivation than E or F prostaglandins and could have a longer half-life in the circulation (27–29).

The release of prostaglandins into the venous effluent from other organ systems and subsequent return to the pulmonary vascular bed could markedly affect pulmonary function. Prostaglandins that arrive by way of the circulation would be expected to affect mainly the precapillary side of the pulmonary circulation because transit across the lung results in almost complete inactivation of E and F type compounds. In contrast, release of prostaglandins from the lung itself could be expected to affect mainly the postcapillary vessels (pulmonary lobar veins).

Marked physiologic and anatomic differences exist between various vascular beds in the body. The physiology of the pulmonary vascular bed is more complicated and difficult to study than peripheral vascular beds such as the skin, skeletal muscle, spleen, or intestine. The major problems in studying the pulmonary circulation as pointed out by Waaler (34) are consequences of the special structure of the lungs and their position within the chest in which respiratory movements impose considerable rhythmic changes in pressure and volume. Thoracotomy could alter several parameters including vascular geometry, transmural vascular pressure, and vascular volumes (34). In addition, alterations in cardiac function will influence the vascular pattern of the pulmonary vessels through which the entire output of the right ventricle must pass (34).

The demonstration of an active response of the pulmonary vascular bed requires experimental conditions that exclude the participation of passive factors that may alter or obscure the direct effects of a prostaglandin on the pulmonary vessels.

The effect of changes in pulmonary blood flow on vascular resistance in the lung is shown in Figure 1. It is apparent that small changes in blood flow, especially in the physiologic range of flow where the curve is steepest, can effect large changes

in pulmonary vascular resistance. The relationship between blood flow and vascular resistance exists in a part of one lobe, in one or two lobes, or in the entire pulmonary vascular bed (35, 36). It has been well documented that the prostaglandins can elicit marked changes in cardiac output, and because blood flow is an uncontrolled

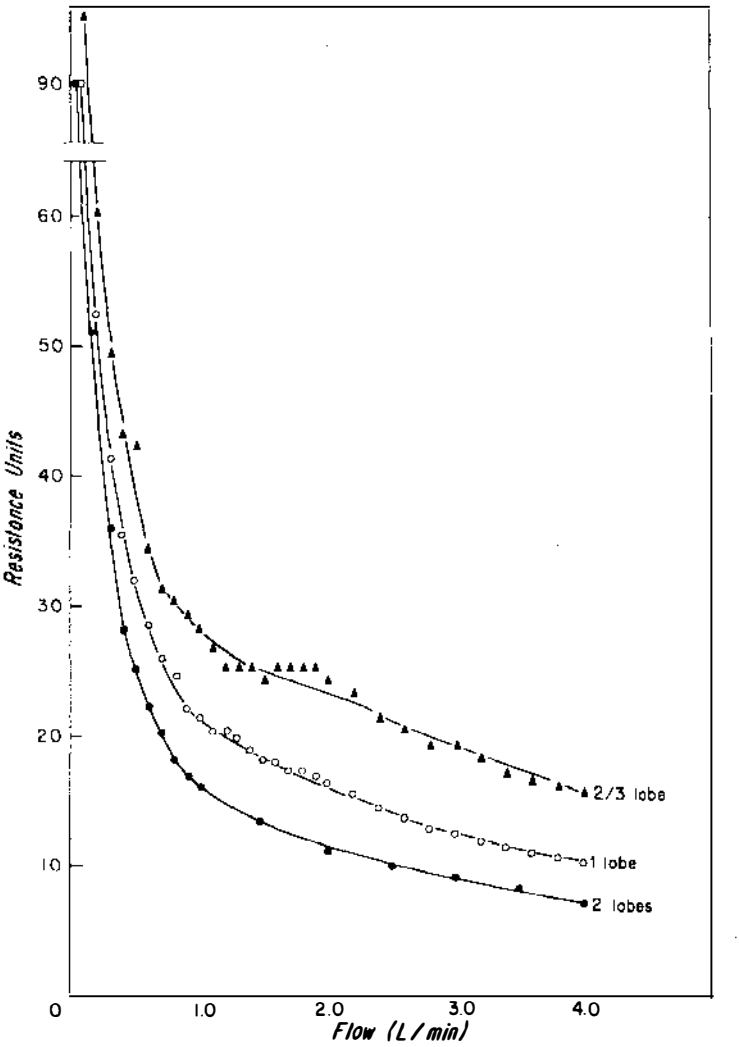

Figure 1 Relationship between blood flow and pulmonary vascular resistance in the intact dog. The flow-resistance curve was determined in two thirds of a lung lobe, in one lobe, and in two lobes. This figure shows that resistance is inversely related to flow and that this relationship is nonlinear.

variable, it would be difficult if not impossible to sort out the direct effects of these naturally occurring lipids on the pulmonary vascular bed in the intact animal (12, 13, 35, 36).

Changes in bronchomotor tone, rate, rhythm, and volume of respiration and alterations in the pattern of bronchial blood flow may modify the response of the pulmonary vessels to a prostaglandin (36). The effect of changes in bronchomotor tone are of particular importance in regard to the actions of prostaglandins on pulmonary vessels because these substances have been shown to have marked effects on airway smooth muscle (12, 13, 37). It has been documented that there are anatomic and physiologic communications between the high pressure systemic circulation and the low pressure pulmonary vascular bed by way of the bronchial circulation that subserve a nutritive function for the lung itself (36). Prostaglandins, especially E and A type compounds, can produce marked changes in systemic arterial pressure which may in turn alter bronchial blood flow. Most of these considerations are circumvented when the lung is removed from the body and perfused in a chamber at a constant rate of flow. In the isolated perfused organ most parameters can be carefully controlled, but the experimental conditions are far removed from the normal physiologic conditions in the intact animal, making interpretation of the biological significance of such results difficult.

Another experimental approach that was used to study the effects of the prostaglandins on the pulmonary circulation has been to perfuse a lobe of the lung in the intact animal (38). A technique has been developed whereby right heart and transseptal catheterization techniques were used to isolate and perfuse the left lower lung lobe at a controlled rate of flow in the lightly anesthetized, intact spontaneously breathing dog (38). When blood flow is maintained at a constant rate, the gradient in pressure between the lobar artery (measured at the tip of the perfusion catheter) and the left atrium is proportional to the resistance to the flow of blood across the lung. Drugs that actively constrict pulmonary vessels would be expected to increase the pressure gradient across the lung, and agents that actively dilate the pulmonary vascular bed would be expected to decrease this gradient. The procedure by which flow is maintained constant in the intact dog is illustrated in Figure 2, and this technique has been modified for use in the intact swine and lamb (39, 40). In this procedure the anesthetized animal is strapped to a fluoroscopic table, and a specially designed 20F balloon double lumen perfusion catheter is introduced from the external jugular vein and passed into the right ventricle and out into the artery of the left lower lung lobe under fluoroscopic guidance. The balloon on the perfusion catheter is distended with Hypaque® to isolate the left lower lobe from the rest of the pulmonary circulation. The vascular isolation is confirmed in two ways: 1. contrast media (Hypaque), is conspicuously absent from the lower lobe when injected into the main pulmonary artery, and 2. pressure in the lobar artery distal to the balloon approaches pressure in the left atrium when blood flow to the isolated lung is arrested by stopping the pump. After the animal has been heparinized, the vascularly isolated lobe is perfused at controlled flow with blood withdrawn from the right atrium. Both piston and roller pumps can be used so that vascular patterns may be studied under conditions of pulsatile or steady flow. The pumping rate is

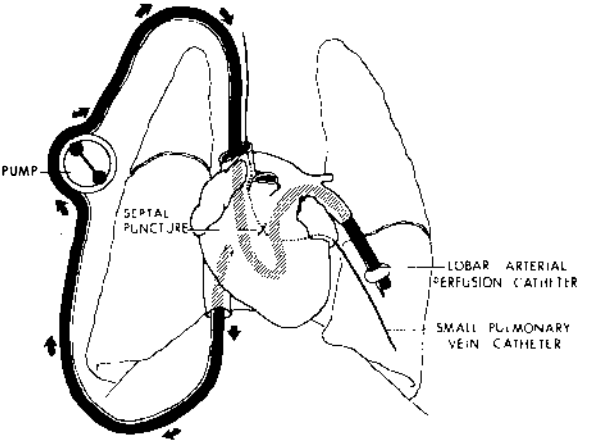

Figure 2 Diagram of the right heart catheterization procedure. The lobar arterial perfusion catheter is introduced from the external jugular vein and passed through the right heart and into the artery of left lower lobe. The balloon on the perfusion catheter isolates the left lower lobe from the rest of the pulmonary circulation, and the lobe is pump-perfused with blood withdrawn from the right atrium. Pressure is monitored in the lobar artery through a catheter with its tip positioned 2 cm distal to the balloon. Pressures are also measured in a small pulmonary lobar vein and in the left atrium with transseptally placed catheters. This technique has been used in the intact dog, swine, and lamb.

adjusted so that pressure in perfused lobar artery approximates pressure in the main pulmonary artery and thereafter is unchanged during an experiment. Although the left lower lobe is vascularly isolated, its autonomic innervation remains intact as evidenced by the response of the lobe to direct electrical stimulation of its sympathetic nerve supply (41–43).

Vascular pressures are also measured in the left atrium and in a small pulmonary lobar vein with transseptally placed catheters. The total pressure gradient across the lung as well as gradients from lobar artery to small lobar vein and from small lobar vein to the left atrium can be measured. By analyzing these gradients it is possible to identify the site of action of a particular agent or prostaglandin in the lung.

Through use of this preparation the direct effects of $PGF_{2\alpha}$ on canine pulmonary vascular bed were first documented in 1969, more than 30 years after Euler described the pharmacologically active principles of seminal fluid (38). These studies demonstrate that $PGF_{2\alpha}$ produces striking increases in pulmonary vascular resistance in the intact dog under conditions of controlled blood flow when infused into the lobar artery (38, 44). Because the increase in resistance was associated with a marked increase in the pressure gradient between the lobar artery and the lobar small vein and between the small vein and the left atrium, vasoconstriction occurred in lobar veins and upstream to the small veins presumably in lobar arteries. These studies indicate that $PGF_{2\alpha}$ is one of the most active pulmonary pressor substances in the intact dog, and are surprising in view of the rather weak vasoconstrictor

activity of this lipid in several peripheral vascular beds in this species (45–47). Nakano & Cole reported that $PGF_{2\alpha}$ increased pulmonary arterial pressure in the open chest dog when cardiac inflow was maintained constant (48). $PGF_{2\alpha}$ has also been shown to increase right ventricular systolic pressure in the cat and calculated pulmonary vascular resistance in the dog, unanesthetized calf, and isolated calf lung (18, 49–52).

The effects of $PGF_{2\alpha}$ on the pulmonary circulation have been studied in the intact swine and lamb using similar right heart and transseptal catheterization techniques (39, 40). In both species $PGF_{2\alpha}$ was found to increase pulmonary vascular resistance under conditions of controlled blood flow (39, 40). In both species this prostaglandin was at least as active as in the dog. However, the site of action was found to be species dependent. In the dog both veins and upstream vessels were constricted, whereas only upstream vessels, presumably lobar arteries, were constricted in the swine and lamb (39, 40). The effects of $PGF_{2\alpha}$ on the swine and lamb lung are interesting in view of the report that the lung of both species contains large amounts of this substance (18).

Because $PGF_{2\alpha}$ is both a vasoconstrictor and a bronchoconstrictor agent, the possibility exists that changes in bronchomotor tone may participate in the response of the pulmonary vascular bed to this substance. This possibility was evaluated by comparing pulmonary vascular responses to $PGF_{2\alpha}$ in normal respiring and atalectatic lung lobes in the intact animal (53). Results of these studies demonstrate that in the intact dog, the pulmonary pressor response to $PGF_{2\alpha}$ is not different in normal and atalectatic lungs (53). It was therefore concluded that in the dog at least, changes in bronchomotor tone or in rate, rhythm, and volume of respiration contribute little, if anything, to the pressor response to this lipid. In other studies the effects of $PGF_{2\alpha}$ on the pulmonary vascular bed were compared in experiments in which the lung was perfused with blood or with dextran in the intact dog (54). In these experiments low molecular weight dextran was buffered to pH 7.3–7.4, heated to 37°C, and perfused at a controlled rate of flow. The perfused dextran was removed from the left lower lobe by way of an 18F withdrawal catheter which had been placed transseptally in the vein draining the lobe. The pulmonary pressor response to $PGF_{2\alpha}$ was similar in blood and dextran-perfused lobes so that platelet aggregation and interaction or release of endogenous substances from elements in blood are probably not involved in the dog. In these experiments the prostaglandin was infused into the lobar artery, and because the dextran perfusate, infused prostaglandin, and metabolites were removed from the animal by way of a withdrawal catheter in the lobar vein, little of the $PGF_{2\alpha}$ would recirculate. This would exclude the $PGF_{2\alpha}$ from the systemic circulation and subsequently from the bronchial circulation. Because pulmonary vascular responses to $PGF_{2\alpha}$ are not different in experiments in which dextran perfusion was used, it is unlikely that changes in bronchial hemodynamics are involved in the response to this substance.

When taken together, these findings indicate that in the dog $PGF_{2\alpha}$ is a potent naturally occurring pulmonary pressor substance that increases pulmonary vascular resistance by constricting lobar veins and vessels upstream to the small veins. In addition, passive factors such as alterations in bronchomotor tone, rate, rhythm, and

volume of respiration, or the bronchial circulation contribute little, if anything, to the response of the canine pulmonary vascular bed to this substance. Furthermore, the canine pulmonary vascular bed is exquisitely sensitive to $PGF_{2\alpha}$ in that responses can be obtained with concentrations as low as $10^{-12}M$ in lobar arterial blood. The effects of $PGF_{2\alpha}$ on the pulmonary circulation are not due to the interaction of this substance with formed elements. These results suggest that if $PGF_{2\alpha}$ were released from peripheral organs or from an intrapulmonary site it might be expected to have marked effects on pulmonary blood volume, the distribution of blood flow within the lung, capillary dynamics, and bronchomotor tone. Because it has been documented that PGF-like substances are released from the lung during anaphylaxis, embolization, distention, and pulmonary edema (23–26, 55–57), it would not be surprising if some of the clinical symptoms and complications accompanying such conditions were caused by prostaglandins.

In reference to disease states that affect the airways, Mathé and co-workers have shown that patients with bronchial asthma are exquisitely sensitive to the bronchoconstrictor effect of $PGF_{2\alpha}$ (58–60). In this remarkable study, patients with bronchial asthma were found to be about 8000 times more sensitive to inhaled aerosols of $PGF_{2\alpha}$ than healthy control subjects. In the asthmatics $PGF_{2\alpha}$ often produced symptoms resembling allergen-provoked asthmatic attacks (58–60). In contrast, the asthmatic patients were only about ten times more sensitive to the bronchoconstrictor effect of histamine. The authors concluded that locally formed $PGF_{2\alpha}$ may play an important role in the pathogenesis of bronchial asthma (58–60).

It has been reported that the major route of metabolism for prostaglandins in the lung is the oxidation of the 15-hydroxyl group by the enzyme prostaglandin dehydrogenase (30–33). The metabolic products formed in the lung are dependent on 15-dehydrogenation so that alternate routes such as β-oxidation are probably not present in the lung (30–33). Because the 15-methyl analog of $PGF_{2\alpha}$ is not a substrate for the dehydrogenase, the analog should be a more potent pulmonary pressor substance than the parent compound (61). In this regard it has been reported that 15-methyl $PGF_{2\alpha}$ produced a marked increase in calculated pulmonary vascular resistance in the open chest dog and that the duration of the pressor response was much greater when compared to the parent compound (61). The effects of the analog on the pulmonary circulation have been examined in the intact dog under conditions of controlled flow (62). In this preparation, the analog was found to produce striking increases in pulmonary vascular resistance when infused into the lobe at rates as low as 1 μg/min. This infusion rate was calculated to achieve a final concentration of $1 \times 10^{-10}M$ in lobar arterial blood. The increase in pulmonary vascular resistance in response to this analog was brought about by vasoconstriction in lobar veins and upstream vessels. When compared to the parent compound, the analog was found to be one to three times more potent, making it the most powerful pressor substance ever studied in the intact dog (62). These results are in support of the conclusion that dehydrogenation is an important mechanism for inactivation of F type prostaglandins in the lung. The effects of 15-methyl $PGF_{2\alpha}$ on the pulmonary circulation were similar in experiments in which the lung was perfused with blood or with dextran (62).

The effects of $PGF_{1\alpha}$, $PGF_{1\beta}$, and $PGF_{2\beta}$ on the pulmonary circulation have been investigated (63, P. J. Kadowitz, in preparation). In the intact dog $PGF_{1\alpha}$ increases pulmonary vascular resistance by constricting lobar veins and upstream vessels (63). In this respect, it is nearly as potent as $PGF_{2\alpha}$. Hence a decrease in the number of double bonds on the side chain of PGF molecule confers little change in biological activity in the pulmonary circulation. However, when $PGF_{2\beta}$ and $PGF_{1\beta}$ were studied, they were found to be devoid of significant vascular activity in the lung (P. J. Kadowitz, in preparation). Hence a change in configuration of the hydroxyl group at carbon nine abolishes the pressor activity of PGF in the lung. It should be noted that all naturally occurring prostaglandins have the α configuration (12, 13).

The effects of E type prostaglandins on the pulmonary circulation have been investigated in a variety of species including dog, rabbit, sheep, swine, and calf (38–40, 43, 44, 48–54, 64). The effects of PGE_1 on pulmonary vascular resistance were evaluated in the isolated blood-perfused rabbit lung (64). In these experiments the isolated rabbit lung was ventilated with 5% CO_2 in air, and left atrial pressure was kept constant. PGE_1 in doses from 1 to 10 μg when injected directly into the perfusion circuit decreased perfusion pressure in the pulmonary artery (64). Relatively large variations in response were seen, and the maximum decrease in pressure was about 5 mm Hg. The decrease in pulmonary vascular resistance in the rabbit was not blocked by propranolol, a beta adrenergic blocking agent (64). It was concluded that PGE_1 was a fairly potent vasodilator in the isolated rabbit lung (64). PGE_1 was also found to decrease pulmonary arterial pressure in the open chest dog when cardiac output was kept constant with a pump (48).

The effects of PGE_1 on the pulmonary circulation were investigated in the intact dog under conditions of controlled blood flow (38, 44). In the intact dog PGE_1 decreased pulmonary vascular resistance by dilating lobar veins and upstream vessels, presumably lobar arteries (38, 44). The decrease in pulmonary vascular resistance in the intact dog and isolated rabbit lung were similar in magnitude, and in the intact dog PGE_1 was one of the most potent pulmonary vasodilator agents studied (38, 44). The effects of PGE_1 on pulmonary vascular resistance have been studied in the isolated perfused calf lung (51). PGE_1 was found to be a potent vasodilator agent in the isolated calf lung (51). The actions of PGE_1 on the pulmonary circulation have been investigated in the intact dog in which the lung was perfused with dextran rather than blood (54). In the dextran-perfused lung PGE_1 was found to be a potent dilator agent, and the magnitude of response was similar in blood- and dextran-perfused lungs (54).

The effects of PGE_1 have been investigated in the swine and lamb under conditions of controlled flow using right heart and transseptal catheterization techniques. In both the intact swine and lamb, PGE_1 was found to be a potent vasodilator agent. In both species the decrease in pulmonary vascular resistance was brought about by vasodilation in vessels upstream in the precapillary bed (39, 40). Although PGE_1 is a potent pulmonary vasodilator agent in the intact dog, swine, and lamb, the site of action is different since in the dog both lobar veins and upstream vessels were dilated (38, 44).

The effects of PGE_2 on the pulmonary circulation have been investigated in the dog, swine, lamb, and calf (50–52, 65). PGE_2 has been shown to increase calculated pulmonary vascular resistance in the calf and dog (50, 52). However, this agent has been reported to decrease pulmonary vascular resistance in the isolated perfused calf lung (51). The reason for the apparent difference in results in the unanesthetized calf and isolated calf lung are unknown (50, 51). In the intact dog PGE_2 increased pulmonary vascular resistance by constricting lobar veins and to a lesser extent vessels upstream in the precapillary bed (65). In terms of its ability to increase pulmonary vascular resistance in the intact dog under conditions of controlled blood flow, PGE_2 is approximately one tenth as potent as $PGF_{2\alpha}$. PGE_2 also increased pulmonary vascular resistance in the intact swine and lamb (65). In both species, the increase in resistance was the result of vasoconstriction in the upstream vessels. PGE_2 was much more potent as a pulmonary pressor substance in swine and lamb than in the dog (65). It is of interest to note that in most peripheral vascular beds PGE_2 is a potent vasodilator agent, whereas in the pulmonary vascular bed of most species studied it is a vasoconstrictor agent (12, 13, 65). The reason for the apparent difference in effect in the pulmonary and peripheral vascular beds is unknown; however, several other naturally occurring substances, including bradykinin, histamine, and acetylcholine, are similar to PGE_2 in that they cause pulmonary vasoconstriction and systemic vasodilatation (66, 67). It has been reported that PGE_2 can cause platelet aggregation (68, 69). The possibility exists that PGE_2 may increase pulmonary vascular resistance by mechanically obstructing the pulmonary vascular bed with platelet aggregates. This hypothesis was tested in experiments in the intact dog in which the lung was perfused with dextran. In the dextran-perfused lung PGE_2 was found to increase pulmonary vascular resistance (65). Since platelets are not present in the perfusion media in these experiments and the pressor response to PGE_2 was as good if not better, it is unlikely that platelet aggregation, or for that matter interaction with any formed element, is involved in the response of the pulmonary vascular bed to PGE_2 (65).

As pointed out earlier, both PGE_1 and PGE_2 are potent peripheral vasodilator agents and PGE_1 is also a pulmonary vasodilator agent, whereas PGE_2 is a mild pressor agent in the lung (65). In addition, the ability of PGE_2 to increase pulmonary vascular resistance was not the result of platelet aggregation in the lung. These data indicate that change in number of double bonds in the side chain has a remarkable effect on the biological activity of E type prostaglandins in the pulmonary vascular bed.

The effects of the 15-methyl analog of PGE_2 have been studied in the open chest dog (61). This substance was found to elicit marked increases in pulmonary arterial pressure, and since cardiac output was either unchanged or decreased, calculated pulmonary vascular resistance was increased greatly (61). The effects of the 15-methyl analog of PGE_2 were studied in the intact dog under conditions of controlled blood flow (62). These studies demonstrate that the analog is a very potent pulmonary pressor substance. At a final concentration of $10^{-8}M$ in lobar arterial blood, 15-methyl PGE_2 was found to double pulmonary vascular resistance in the intact dog (62). This increase in pulmonary vascular resistance was due to vaso-constric-

tion in lobar veins and in upstream vessels (62). In this preparation, the analog was found to be 10 to 30 times more potent than the parent compound, and as potent a pressor substance in the lung as any prostaglandin studied (62). The effects of 15-methyl PGE_2 on pulmonary vascular resistance were similar in experiments in which the lung was perfused with dextran. Hence it is doubtful that platelet aggregation is important in the pressor response to the analog (62).

The effects of E and F type prostaglandins on the cardiopulmonary system have received considerable attention in recent years; however, very little is known about the effects of A type compounds on the pulmonary vascular bed. The effects of PGA_1 and PGA_2 on pulmonary circulation have been investigated in the intact dog under conditions of controlled blood flow (63). In the intact dog, PGA_1 decreased pulmonary vascular resistance by dilating lobar veins and upstream vessels (63). In terms of relative potency, PGA_1 and PGE_1 are quite similar, and both compounds are among the most potent pulmonary vasodilator substances in the dog (63). In contrast, PGA_2 was found to be a mild pressor substance in the canine lung (63). The increase in pulmonary vascular resistance in response to this substance was brought about by vasoconstriction in lobar veins and to a lesser extent in upstream vessels (63). The effects of PGA_2 on the pulmonary vascular bed were not due to changes in physical or chemical properties of blood or its formed elements, because the pressor response to this lipid was, if anything, more pronounced when the lung was perfused with dextran (63).

The natural occurrence of A type prostaglandins has been questioned, and it has been suggested that PGA_2 may be derived from the dehydration of PGE_2 during isolation and purification (70, 71). However, the answer to this question must await further study in view of the observation that substances that are immunoreactive with antibodies to A type prostaglandins have been found in fairly high titer in peripheral plasma of normal adults (72, 73). Prostaglandins of the A series are more resistant to inactivation in the lung than E and F type compounds and could theoretically act as circulating hormones (27–29). In this regard they could serve as circulating antihypertensive hormones since both PGA_1 and PGA_2 are potent vasodilator substances in the peripheral circulation (12, 13, 75, 76). The alteration in biological activity in the lung with a change in double bond in the side chain is similar for A and E type prostaglandins.

The effects of B type prostaglandins on the cardiopulmonary system have received even less consideration than A type compounds. It had been thought that B type compounds were inactive because they had little or no effect on systemic arterial pressure in the rat or on hindlimb vascular resistance in the dog (45, 76). However, recent studies indicate that PGB_2 possesses significant vasoconstrictor activity in the rat pancreas and dog cutaneous vascular bed (77–79). It has been reported recently that PGB_2 increases pulmonary arterial pressure and decreases systemic arterial pressure in the dog (78). Although the increase in pulmonary arterial pressure was marked, the effect of this substance on the pulmonary vascular bed was uncertain since pulmonary blood flow was not measured in these experiments (78).

The effects of B type prostaglandins on the pulmonary circulation have been investigated in the intact dog (80). Under conditions of controlled flow in the dog both PGB_1 and PGB_2 were found to increase pulmonary vascular resistance b

constricting lobar veins and vessels upstream to the small veins, presumably the lobar arteries (80). In the intact dog in regard to its pulmonary pressor activity, PGB_2 is about ten times more active than PGB_1 and is very nearly as active as $PGF_{2\alpha}$. Hence PGB_2 is one of the most potent pulmonary pressor substances in the canine lung. Prostaglandins of the B series were thought to be inactive metabolites of A type compounds (81). However, it is apparent that B type prostaglandins are not inactive metabolites but possess marked pressor activity in the lung, spleen, and cutaneous vascular bed (77–80). It is possible that the pressor activity of PGA_2 may result in part from the conversion of PGA_2 to PGB_2 in the lung. There is little current information about the natural occurrence and distribution of B type prostaglandins in the body although it is known that they are present in large quantities in human seminal plasma (82). Although the physiologic role of B series prostaglandins is unknown at the present time, their marked pressor activity in the lung, pancreas, and cutaneous vascular bed may suggest a role in physiologic and pathophysiologic processes in these organs.

The effects of prostaglandins E_1, E_2, A_1, A_2, $F_{1\alpha}$, $F_{2\alpha}$, $F_{2\beta}$, and the 15-methyl analogs of E_2 and $F_{2\alpha}$ on pulmonary vascular resistance in the intact dog under conditions of controlled blood flow are summarized in Table one. In addition, their relative activities as pulmonary vasoconstrictor or vasodilator agents have been compared with other naturally occurring substances such as norepinephrine, serotonin, angiotensin, histamine, acetylcholine, and bradykinin. Table 1 shows that prostaglandins are among, if not the most potent vasoactive substances in the lung and that they exhibit a remarkable spectrum of activity on pulmonary circulation. This spectrum extends from marked vasodilation with PGE_1 to inactivity with $PGF_{1\beta}$ to striking pressor activity with $PGF_{2\alpha}$. Therefore, changes in configuration of the hydroxyl group at the nine carbon alters the activity of F type compounds in a marked fashion. Changes in number of double bonds in the side chain have striking effects on the biological activity of A, E, and B prostaglandins but not on the activity of F type compounds. In addition, 15-methylation greatly enhances the pressor activity of PGE_2 and to a lesser extent the pressor activity of $PGF_{2\alpha}$. Since the 15-methyl analogs are not substrates for the enzyme prostaglandin dehydrogenase, the enhanced biologic activity suggests that dehydrogenation is an important pathway for inactivation in the lung.

The great biological activity along with their natural occurrence, synthesis, release, and inactivation suggest that these lipids may have important implications in the regulation of the pulmonary circulation and in the pathogenesis of a number of disease states including pulmonary hypertension, pulmonary edema, anaphylaxis, and bronchial asthma.

EFFECTS OF PROSTAGLANDINS ON ISOLATED INTRAPULMONARY ARTERY, VEIN, AND BRONCHUS

Several investigators have studied the action of prostaglandins on isolated airway smooth muscle. Most of these studies employed tracheal or extrapulmonary bronchial muscle preparations. Horton (84) has reviewed the early literature. In vitro, tracheal muscle preparations from various species (cat, monkey, rabbit, guinea pig,

Table 1 Effect of prostaglandins A, E, F, and B and certain other naturally occurring substances on the pulmonary circulation in the intact dog under conditions of controlled blood flow[a]

Agent	Effect	Relative activity	Reference
$PGF_{2\alpha}$	constriction	++++	38, 44, 54
$PGF_{2\beta}$	none	0	A
$PGF_{1\alpha}$	constriction	+++	63
$PGF_{1\beta}$	none	0	A
PGE_1	dilatation	++++	38, 44, 54
PGE_2	constriction	+	65
PGA_1	dilatation	++++	63
PGA_2	constriction	+	63
PGB_1	constriction	++	80
PGB_2	constriction	++++	80
15-Methyl PGE_2	constriction	++++	62
15-Methyl $PGF_{2\alpha}$	constriction	++++	62
Serotonin	constriction	++++	66
Bradykinin	constriction	+	67
Norepinephrine	constriction	++	83
Isoproterenol	dilatation	+++	66
Histamine	constriction	+	66
Acetylcholine	constriction	+	66
Angiotensin	constriction	++	83

[a] ++++ = greatest activity, + = least activity, 0 = no activity, A = P. J. Kadowitz, unpublished observation.

ferret, sheep, and pig) are relaxed by PGE_1 when adequate tone is present, usually achieved by prior addition of acetylcholine (ACh) or $BaCl_2$. Cat tracheal muscle contracting to ACh was also relaxed by PGE_2, PGE_3, and $PGF_{1\alpha}$ (85). Human bronchial muscle was relaxed by PGE_1 and PGE_2 but was contracted by $PGF_{2\alpha}$ (84, 86).

Recently, calf isolated bronchial muscle has been found to relax when exposed to PGE_1 provided the muscle tone was enhanced by ACh; $PGF_{2\alpha}$ had no effect (51). PGE_1 has been shown to inhibit contractions of dog isolated trachea induced by a variety of agents including serotonin (5-HT) and ACh but not that due to a high K^+ depolarizing solution. Methysergide blocked the inhibitory effect of PGE_1, prompting the suggestion that the 5-HT and PGE_1 receptors in this preparation are near one another (87).

Although a good deal of information is known concerning the actions of prostaglandins on many isolated vascular smooth muscle preparations (84), little work has been done on isolated pulmonary vascular smooth muscle. That which has been reported dealt with extrapulmonary vessels. Lewis & Eyre (51) found that $PGF_{2\alpha}$ elicited dose-related contraction of calf pulmonary artery and vein, whereas PGE_1

and PGE_2 relaxed both vessels submaximally contracted with histamine (His), 5-HT, or ACh.

Others' successful use of isolated smooth muscle segments for studying prostaglandin pharmacology (51, 84–90) prompted us to attempt to assay the effects of prostaglandins on isolated intrapulmonary smooth muscle.

A search of the literature revealed a paucity of previous reports concerning the action of any pharmacological agents on these preparations. The few studies (36, 91–100) dealing with intrapulmonary smooth muscle usually assessed the effects of the more classic agents, ACh, norepinephrine (NE) and epinephrine, His, and 5-HT. Even for this limited number of agonists, reports were scanty, lacking in quantitative detail, often contradictory, and sometimes disappointingly sparse in methodology.

Accordingly, we undertook the study by first determining to our own satisfaction the mechanical responses of isolated intrapulmonary vessels and airways to classic drugs.

Vessel and airway segments 2–6 cm long were carefully dissected from canine lung lobes. We initially utilized segments of 2–5 mm diam but have since studied both larger and smaller segments. The segments usually were from main branches of the main lobar vessels and bronchus. The degree of branching is such that the segments contain numerous holes where branches were originally attached. The adhering tissue was carefully stripped away and a helical strip was prepared from each segment (101), care being taken to cut through the holes whenever possible. The central section of the strip contained some holes but their number was minimized by the preparative technique. The strips were about 3–5 mm wide. The same helical strip could sometimes be divided into several smaller strips (1.5–3 cm long) and responses of successive portions of the same segment assayed.

Silk ties were attached to either end of each strip which was then mounted for isometric tension recording in a 10 ml muscle bath. The strip was always kept moist during the preparative procedure and once in the bath was surrounded by a bathing medium consisting of a physiological salt solution. In the initial studies this solution was composed of as few salts as possible: NaCl (125 m*M*), KCl (2.7 m*M*), $CaCl_2$ (1.8 m*M*), and Tris (23.8 m*M*). Glucose (11 m*M*) was also present. The temperature was maintained at 37°C and the pH at 7.4. Tissues responded well and in a reproducible fashion even after 8–12 hr in such a solution, bubbled continuously with pure O_2. The advantage of using such a bathing medium initially was that the effects of H^+, Ca^{2+}, Mg^{+2} bicarbonate, CO_2, phosphate salts, etc could be assessed later after control responses had been determined.

Isometric tension changes were detected by a Grass force-displacement transducer and recorded on a Grass polygraph. Isotonic shortening was also measured in some tissues by means of an ink-writing lever system and standard Bird kymograph.

The initial studies were concerned with defining the optimal loading forces. All preparations responded well to 5-HT under the appropriate stretching forces. Optimal loads were 4 g for arterial strips, 3 g for venous strips, and 2 g for bronchial strips. When these loads on the vascular strips were equated to in vivo wall tension, it was found that the latter were approximated rather well. Strips under these resting

loads stretched somewhat initially and then maintained a constant resting length and tension output. They would respond to 5-HT for many hours in a constant, reproducible fashion (Figure 3).

Subsequent experiments with other agonists were performed (102, 103). Norepinephrine (NE), histamine (His), and epinephrine (Epi) also contracted the vessel strips in a dose-related fashion. Maximum active tension output was about 30 g/cm^2. His contracted airway strips, though not as well as ACh. The latter drug contracted venous strips well. ACh relaxed arterial strips in a dose-related fashion. Subsequently it was found that smooth muscle depressant drugs like nitroglycerin and sodium nitrite relaxed vascular tissue. Tension on the strips often decline as much as 0.5 g or more in the presence of these agents. Bronchi did not respond to smooth muscle depressants unless they were already in a partially contracted state as when a submaximal level of ACh was present. Under these conditions, the beta agonist, isoproterenol, was especially effective

In another study, we found that stimulatory agents could elicit isotonic shortening of helical strips of canine intrapulmonary arteries, veins, and bronchi (104). Since these preparations were under loads approximating in vivo wall tensions, the data suggest that the portions of intrapulmonary vasculature and airway from which the strips were obtained alter their lumen size in vivo when appropriately stimulated.

With responses to the classic pharmacological agents established, the effects of a number of prostaglandins were determined (105, 106). Since the classic agents had exhibited maximum responsiveness at concentrations of 10^{-9}–$10^{-5}M$, it was decided to study the prostaglandins over the same range of concentrations. Preliminary studies revealed that the quantity of the ethanol vehicle required to maintain the prostaglandin in a soluble state in the bathing medium had no effect
ical activity of the strips.

Dose-related contractile responses occurred upon introduction of almost all the prostaglandins to the bathing solution surrounding venous strips (Table 2). PGB_1, PGB_2, $PGF_{2\alpha}$, and 15-methyl $PGF_{2\alpha}$ were especially active being equal to or more effective than an equivalent concentration of NE. PGE_1 induced some degree of relaxation which was dose-related. Responses of arterial strips showed much more variability to the various prostaglandins. None were very active in the arterial segments except PGA_1 and PGA_2. These prostaglandins were especially interesting in that the presence of a tungsten lamp (75 watt) shining on the bath seemed to diminish the relaxant effect on the arteries and enhance the stimulatory action on the veins. The responses to none of the other prostaglandins appear to be altered by light. As yet no explanation for this behavior has been found, although partial conversion of PGA to PGB might account for these findings (107). At any rate, the effect of light to enhance the contractile response to the PGAs is opposite from the reported action of light to induce relaxation of rabbit aortic strips (101) and probably does not depend on the same underlying mechanisms.

Fewer studies have been done to date on canine bronchial strips. PGA_1, PGA_2, PGB_1, PGE_2, and $PGF_{1\alpha}$ (10^{-8}–$10^{-5}M$) appear to be without effect in the unstimulated muscle. $PGF_{2\alpha}$ occasionally elicited a small degree of contraction but

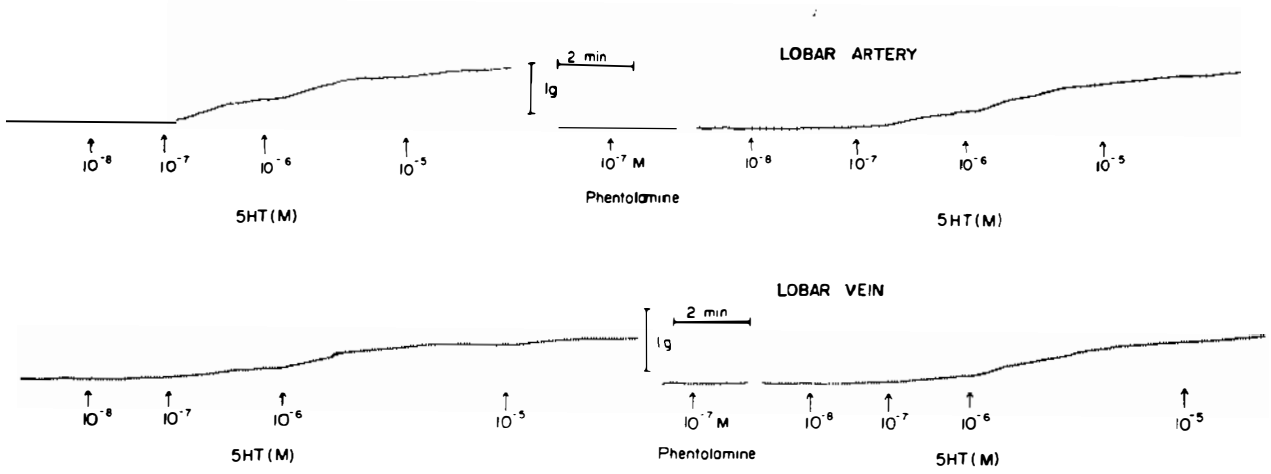

Figure 3 Responses of canine isolated intrapulmonary artery (*top*) and vein (*bottom*) to 5-HT (10^{-8}–10^{-5} *M*). Control responses are illustrated on the left. Responses obtained in the presence of the alpha adrenergic blocking agent, phentolamine (*right*), were not appreciably affected.

Table 2 Effects of various prostaglandins on mechanical activity of canine isolated intrapulmonary vessels[a] (8–15 observations each)

Test substance (10^{-5} *M*)	Artery	Vein
PGA_1	----	++
PGA_2	----	++
PGB_1	0	+++
PGB_2	0	++++
PGE_1	–	–
PGE_2	0	++
$PGF_{1\alpha}$	+	+
$PGF_{2\alpha}$	0	+++
$PGF_{1\beta}$	+	+
$PGF_{2\beta}$	0	+
15-Methyl PGE_2	+	++
15-Methyl $PGF_{2\alpha}$	+	+++

[a] 0 = no significant effect, + to ++++ = minimal to near maximal contraction, – to ---- = minimal to near maximal relaxation.

usually had no effect. PGB_2 contracted canine bronchial strips well and in a dose-related fashion in a few experiments. In a few bronchial strips partially contracted by carbachol, PGE_2 elicited some relaxation. PGB_1 seems to relax slightly at lower levels and to contract the tissue further at $10^{-5}M$. PGA_2 and $PGF_{1\alpha}$ were apparently without effect.

While the majority of our experiments with the prostaglandins have been performed on canine tissues, intrapulmonary arteries, veins, and bronchi from other species have been studied in a limited fashion (Table 3). Arteries from swine contract well to NE and His. Swine veins are especially responsive to 5-HT while bronchi contract well to ACh and His. Neither $PGF_{2\alpha}$ or PGE_1 were active in unstimulated vascular or airway strips from swine. Prestimulated strips have not been tested to determine whether they might possess relaxant properties. Sheep arteries that contract well to NE, His, and 5-HT relaxed when exposed to PGE_1. $PGF_{2\alpha}$ had a stimulatory effect

glandin affected the tone of unstimulated sheep bronchial strips that contracted well to ACh and 5-HT and relaxed when exposed to NE (slightly) and His. PGB_1, PGA_2, and $PGF_{1\alpha}$ had little or no effect on vascular strips.

Intrapulmonary arterial strips from man (108) contracted well when exposed to NE, ACh, 5-HT, and His. $PGF_{2\alpha}$ was also quite active as a stimulatory agent in these tissues, whereas PGE_1 and PGE_2 were less so. Human veins contracted to NE, 5-HT, and His. Again, $PGF_{2\alpha}$ was a stimulatory agent in human veins. PGE_1 relaxed veins from four patients and contracted those from one patient.

Isolated bronchi from man contracted when in contact with ACh. $PGF_{2\alpha}$ had a slight stimulatory action. PGE_1 relaxed human bronchi as did isoproterenol.

Table 3 Effects of certain prostaglandins (10^{-8}–10^{-5} *M*) on mechanical activity of isolated intrapulmonary vessels from various species[a]

Vessel	Species	Response to PGE_1	Response to $PGF_{2\alpha}$
Artery	swine	0	0
	sheep	R	0
	man	sl C	C
	baboon	0 or sl C	C
	chimpanzee	R	C
	monkey	sl C	C
Vein	swine	0	0
	sheep	R	C
	man	sl R	C
	baboon	0 or sl C	C
	chimpanzee	R	–
	monkey	–	sl C

[a] 0 = little or no effect, C = contraction, R = relaxation, sl = slight, – = not tested.

A few experiments have been performed on isolated intrapulmonary arteries and veins from baboon, chimpanzee, and stump-tailed monkey, *Macaca speciosa (arctoides)*. In baboon arteries and veins, $PGF_{2\alpha}$ elicited dose-related contractions. PGE_1 either had no effect or induced slight contraction. In chimpanzee arteries, $PGF_{2\alpha}$ caused contraction at higher concentrations (10^{-6}–10^{-5} *M*). PGE_1 and PGE_2 tended to relax both arteries and veins. Monkey arteries were contracted well by $PGF_{2\alpha}$ and slightly by PGE_1. In monkey veins, ACh and His elicit a small degree of contraction as does $PGF_{2\alpha}$ at higher concentrations (10^{-6}–10^{-5} *M*). Thus in primates, including man, $PGF_{2\alpha}$ consistently contracted intrapulmonary arterial segments and venous strips (when tested). PGE_1's effect seems to be more variable, depending on species and location of the vessel tested.

Our studies with isolated intrapulmonary vessels and airways suggest that these preparations respond to many prostaglandins. With the exception of PGE_1, all the prostaglandins tested contracted canine intrapulmonary veins. In fact, $PGF_{2\alpha}$ usually contracted veins from most species. A wide range of activity occurred in intrapulmonary arteries, some prostaglandins eliciting contraction, others relaxation, and others having no effect. PGE_1 exhibited all these responses, depending on the species tested.

It is interesting to note that in some instances, the response of isolated canine vascular strips in vitro to certain prostaglandins (Table 2) differed from that inferred from the in vivo studies (Table 1). A number of possible differences could account for this. For example, the vessels studied in vitro may differ in location and size from those responsible for the in vivo findings. The intact vasculature is likely to be under the influence of neural, ionic, metabolic, and hormonal factors absent or altered in the bath. Possible metabolic conversion of the prostaglandin is more likely to occur in vivo. The trauma involved in dissecting the vessel segments and preparing helical strips might have changed the tissue's responsiveness. Regardless of these and other variables, results of the in vitro and in vivo studies are generally in agreement.

EFFECTS OF PROSTAGLANDINS ON SYMPATHETIC TRANSMISSION IN THE CARDIOVASCULAR SYSTEM

It is well established that the autonomic nervous system plays an important role in the regulation of the cardiovascular system and that prostaglandins possess the ability to modify transmission between adrenergic nerve endings and the effector site in blood vessels (46, 109–111). The effects of prostaglandins on sympathetic transmission have been reviewed recently by Hedqvist (109) and by Brody & Kadowitz (111). In general, these substances have been found to exhibit a remarkable spectrum of effects on vasoconstrictor responses to both sympathetic nerve stimulation and exogenous NE (46, 109–111). However, it appears that concentration and prostaglandin (PGE_2, etc) as well as organ or vascular bed and species are important factors in determining the effect of the prostaglandins on sympathetic transmission (49, 109–111). For example, PGE_2 has been shown to enhance the response to nerve stimulation at high concentration but to depress the response to both exogenous norepinephrine and nerve stimulation at low concentration in the dog cutaneous vascular bed (112, 113). PGE_2 has no apparent effect on responses to norepinephrine and nerve stimulation in the canine superficial veins or canine spleen but depresses the response to nerve stimulation in the rabbit heart and feline spleen and hindlimb (114–120). Furthermore, PGE_2 inhibits responses to both NE and nerve stimulation in the renal vascular bed of the dog and cat, and in both species, it is more effective in antagonizing responses to nerve released NE (121, 122). In contrast, $PGF_{2\alpha}$ increased the response to nerve stimulation in the cutaneous vascular bed of the dog (123, 124). This increase occurred in the absence of a change in the response to injected NE or tyramine (123, 124). It was therefore postulated that $PGF_{2\alpha}$ enhances the response to nerve stimulation by facilitating the release of transmitter by sympathetic nerve impulses and that this effect is specific for the pool of transmitter liberated by nervous activity because responses to tyramine were not affected (124). In this regard the effects of $PGF_{2\alpha}$ and angiotensin II on adrenergic transmission were qualitatively similar; however, $PGF_{2\alpha}$ was more potent by at least two orders of magnitude (124, 125). In the canine superficial veins, $PGF_{2\alpha}$ enhanced the response to both exogenous NE and nerve stimulation so that the site of action is different in arterial and venous segments in the hindpaw (126). PGA_2 was found to decrease responses to NE and nerve stimulation in the canine hindlimb (74). It has been documented that prostaglandins are released into the venous effluent during nerve stimulation or infusion of vasoconstrictor agents in a variety of organ systems (127–130). The marked effects of the prostaglandins on sympathetic transmission along with their demonstrated release have led to the hypothesis that these substances may function as modulators of the autonomic nervous system (109–111).

The remarkable discovery that nonsteroidal anti-inflammatory agents inhibit prostaglandin biosynthesis permitted this hypothesis to be tested (14, 15). The effects of eicosa 5,8,11,14-tetraynoic acid (ETA), an inhibitor of prostaglandin synthesis, on the vasoconstrictor response to nerve stimulation was evaluated in the isolated perfused cat spleen (131). After administration of ETA the splenic vasoconstrictor response to nerve stimulation and the outflow of NE were increased whereas the

release of PGE-like material was inhibited (131). The observations that PGE_2 inhibits release of transmitter and decreases the response to nerve stimulation whereas ETA enhances the response to nerve stimulation and output of transmitter strongly support the hypothesis that in the spleen prostaglandins modulate the effects of the sympathetic nervous system (109–111). A similar effect has been reported in the cat spleen with indomethacin, another prostaglandin synthetase inhibitor (132). ETA has also been shown to facilitate sympathetic neurotransmission in the isolated rabbit heart (133).

The effects of ETA and indomethacin on responses to NE and nerve stimulation were studied in the dog cutaneous vascular bed (113). In low concentrations neither ETA or indomethacin had a significant effect on the response to NE or to nerve stimulation (113). Higher concentrations of ETA and indomethacin enhanced the response to both exogenous NE and sympathetic nerve stimulation (113). There was a tendency for vasoconstriction to occur in the paw after administration of the synthesis inhibitors (113). It was also shown in these studies that this potentiation was not due to blockade of NE uptake, because in the concentrations employed the synthesis inhibitors had no apparent effect on the uptake of ^{3}H-NE by these vascular segments (113). The results of these experiments are consistent with the hypothesis that in the cutaneous vascular bed, prostaglandins modulate the effects of the sympathetic nervous system (123). However, in the cutaneous vascular bed the site at which this modulatory action occurs is postjunctional because responses to both nerve-released and exogenous NE were enhanced after treatment with the synthesis inhibitors. The increase in vascular resistance after treatment with the synthesis inhibitors suggests that in the basal state (absence of activity in the sympathetic nerves) endogenous prostaglandins may be important in the maintenance of blood flow in the cutaneous vascular bed.

It has been suggested that PGE_2 of renal origin is a determinant of resting blood flow to the kidney (134). This hypothesis is based on the observation that after treatment with indomethacin or meclofenamate there was a marked decrease in renal blood flow and that this decrease in flow was correlated with reduced efflux of a PGE-like substance from the kidney (134). It has also been shown that when the release of prostaglandin-like material from the kidney was abolished by indomethacin the vasoconstrictor effects of angiotensin II on the renal vascular bed were enhanced (135). These results suggest that locally released prostaglandins play a role in the maintenance and regulation of blood flow to the kidney.

The role of renal prostaglandins in autoregulation in the kidney has been investigated (136). Results of these studies suggest that autoregulation in the kidney is mediated by the intra-renal release of a prostaglandin (136).

Results of experiments with inhibitors of prostaglandin synthesis strongly support the hypothesis that prostaglandins are involved in the regulation of blood flow by virtue of their effects on sympathetic transmission and resting blood flow in a number of organ systems. However, most of these interpretations are based on studies which assume that the synthetase inhibitors are devoid of other biological effects and that the observed action is exclusively the result of inhibition of prostaglandin synthesis. However, in several preliminary studies both indomethacin and

meclofenamic acid have been shown to inhibit the enzyme prostaglandin dehydrogenase (16). Therefore the specificity of the synthetase inhibitors remains to be established.

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