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Mechanism and Modification of Bradykinin-Induced Coronary Vasodilation

(prostaglandin/indomethacin/SQ-20881/heart/bradykininase)

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ABSTRACT **In isolated perfused rabbit hearts, bradykinin produced a concentration-dependent decrease in coronary resistance directly associated with biosynthesis and release of prostaglandin-E-like substance. An inhibitor of bradykinin destruction (the nonapeptide SQ-20881) markedly enhanced both the coronary vasodilation and release of prostaglandin-E-like substance produced by cardiac injection of bradykinin. Indomethacin inhibited both the myocardial prostaglandin biosynthesis and the decrease in coronary resistance induced by bradykinin. The demonstration that bradykinin is a potent stimulator of prostaglandin biosynthesis in the heart has implications as to the cause of the afferent cardiovascular reflexes and pain in myocardial infarction and angina pectoris.**

Bradykinin is generated by the action of a proteolytic enzyme upon plasma alpha-2-globulin. Since proteolytic enzymes are ubiquitous in the body, all tissues presumably are capable of releasing bradykinin from plasma (1). Bradykinin has repeatedly been demonstrated to be one of the most potent coronary dilators in isolated perfused mammalian hearts (2) as well as in intact animals (3–5). Indeed, there is evidence to suggest that bradykinin is involved in the local humoral control of coronary blood flow. The bradykinin-induced coronary vasodilation could be the result of either a direct relaxation of coronary vascular smooth muscle or an indirect action by means of an endogenous substance. As to the second possibility, the action of bradykinin on coronary resistance does not appear to be the result of the elaboration of histamine, catecholamines, acetylcholine, or serotonin (2, 3).

To establish that an endogenous substance mediates a physiologic event requires temporal and quantitative correlation between changes in concentration of the putative mediator with changes in functional status of the organ. The concentration of the mediator substance should be proportional to the stimulus (bradykinin in this case) and, in addition, abolition of the synthesis of the mediator should abolish the physiologic action of the stimulus. In view of the previous demonstration that the heart readily synthesizes and releases prostaglandins (6–8), we have investigated the possible involvement of endogenously synthesized prostaglandins in mediating the coronary vasodilation produced by bradykinin in isolated perfused rabbit hearts.

METHODS

Isolated rabbit (New Zealand) hearts were perfused through the aorta with Krebs-Henseleit medium (in an atmosphere of 95% O_2 and 5% CO_2) at a constant flow of 30 ml/min. Changes in perfusion pressure (Statham transducer, Brush recorder) were indicative of alterations in coronary resistance. Ventricular pressure and rate were recorded from a fluid-filled balloon tied into the left ventricle (isovolumic heart).

The coronary venous effluent was continuously and immediately assayed for the presence of vasoactive substances. This was achieved by continuously superfusing a series of isolated assay tissues with the coronary outflow according to the procedure of Vane (9, 10). Rat stomach strip and chick rectum are particularly sensitive to prostaglandins, especially of the E type. Rat colon strips are sensitive to prostaglandin $F_{2\alpha}$ and were employed in the superfusions, but no contractions were elicited on this strip by the coronary effluent from the bradykinin-treated hearts. The substance detected in the coronary effluent in this experiment was most likely prostaglandin E. This conclusion was based primarily on the specificity of the systems used for bioassay (11) and was reinforced by the disappearance of activity after treatment with indomethacin, a specific inhibitor of prostaglandin biosynthesis (12). Confirmation that the prostaglandin-like materials released from the heart, and detected by the bioassay organs, was prostaglandin-E_2 was obtained by acidifying the effluent, extracting it with ethyl acetate, and concentrating the product. It was subjected to thin layer chromatography and the eluted spots were bioassayed.

A mixture of antagonists was added to the coronary effluent below the heart to render the assay tissues insensitive to catecholamines, acetylcholine, serotonin, and histamine (9, 10). The sensitivity of the assay organs to prostaglandins was enhanced by perfusing a solution of indomethacin (kindly supplied by Merck, Sharp, and Dohme) at 10 μg/min across them [i.e., distal to the heart (13)]. The assay organs were calibrated with prostaglandin E_2 standards (kindly supplied by the Upjohn Co.) which were tested at the beginning and the end of each experiment.

Cat jejunum was also employed in the superfusion experiments for detection of bradykinin (9). The cat jejunum was not contracted by prostaglandins (E_1, E_2, or $F_{2\alpha}$) or by angiotensin.

RESULTS

The injection of bradykinin into the aortic cannula proximal to the heart produced a concentration-dependent decrease in coronary resistance (Fig. 1A). The intracoronary injection of bradykinin in the doses tested was without significant effect on heart rate or left ventricular tension. Preinfusion of the heart with the nonapeptide SQ-20881 enhanced the coronary vasodilation produced by bradykinin (Fig. 1A). The enhance-

Abbreviation: PG, prostaglandin.

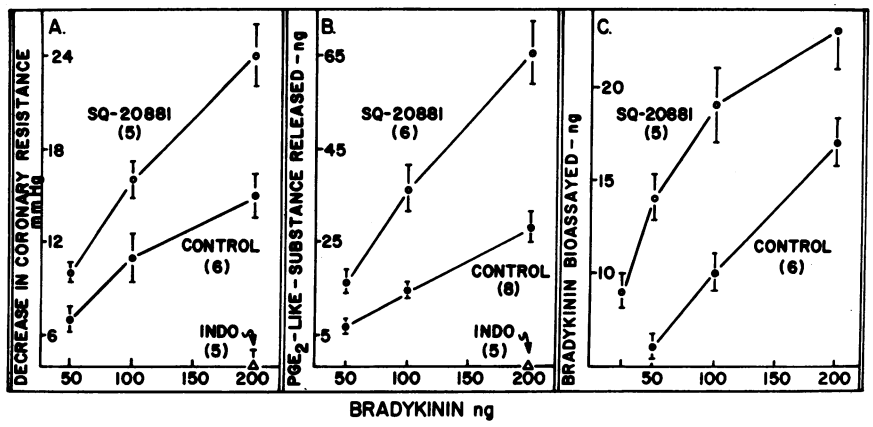

FIG. 1. Comparison of the dose-dependent cardiac effects of bradykinin on: (A) coronary resistance, (B) appearance of prostaglandin-E like substance in the coronary venous effluent, and (C) appearance of intact bradykinin in the coronary venous effluent. The values shown are the mean ± standard error, with the number of hearts at each point indicated in parentheses. Bradykinin was injected as a bolus into the medium as it entered the heart. The prostaglandin-E-like activity in the coronary effluent was calculated from dose–response curves obtained on both rat stomach and chick rectum strips in assays bracketed with standard prostaglandin E_2 dose–response curve. Similarly, bradykinin levels in the coronary effluent were determined by bioassay on cat jejunums by comparison to the direct application of bradykinin standards. SQ-20881 was infused through the heart at 300 ng/ml per min and indomethacin (Indo) was also employed at 300 ng/ml per min. Comparable inhibition of myocardial prostaglandin release was obtained by infusion of meclofenamate.

ment of the response to bradykinin produced by SQ-20881 was reversed upon termination of its infusion. This nonapeptide has previously been demonstrated to potentiate the biological actions of bradykinin by blocking destruction by the pulmonary enzyme bradykininase (14). Thus, the enhancement by SQ-20881 indicates the presence of a potent myocardial bradykininase. On the other hand, pretreatment of the heart with indomethacin, an agent known to inhibit prostaglandin biosynthesis, prevented the coronary vasodilation produced by bradykinin (Fig. 1A). In the presence of indomethacin, very high doses of bradykinin (500 ng or higher) caused only a slight decrease in coronary resistance, which was not enhanced by SQ-20881.

The appearance of prostaglandin-E (PGE)-like substance in the coronary venous effluent was continuously monitored by the superfusion bioassay system. Bradykinin caused a concentration-dependent increase in the release of PGE-like substance from the perfused rabbit heart (Fig. 1B). As in the case of coronary resistance, intracardiac infusion of SQ-20881 markedly enhanced the bradykinin-induced appearance of PGE-like substance, whereas indomethacin abolished the PGE-like substance release by bradykinin (Fig. 1B).

The level of bradykinin in the coronary effluent was measured by bioassay with the cat jejunum. The perfused rabbit heart rapidly and extensively degraded bradykinin; about 90% of the peptide was destroyed after injection of a bolus of bradykinin into the medium as it entered the heart (Fig. 1C). Simultaneous coronary infusion of SQ-20881 protected some of the bradykinin from myocardial destruction, as shown by a 2-fold increase in concentration in the effluent. Thus, the administration of the nonapeptide SQ-20881 led to elevated cardiac bradykinin levels which correlated well with higher levels of prostaglandin E biosynthesis and enhancement of coronary vasodilation.

TABLE 1.

Bradykinin injected, picomoles	PGE-like substance release, picomoles		
	Controls (8)	SQ-20881 (6)	Indomethacin (5)
47	20 ± 3	46 ± 9	0
94	40 ± 3	102 ± 17	0
188	86 ± 11	187 ± 23	0

Relationship between myocardial injections of bradykinin and myocardial prostaglandin biosynthesis. Experimental details are in the legend for Fig. 1.

Evaluation of the data on a molar basis indicated a direct relationship between the concentration of bradykinin injected and the PGE-like substance released (Table 1). In fact, in the presence of SQ-20881, which interferes with myocardial bradykinin destruction, there was a 1:1 ratio between the coronary vasodilation produced and the PGE-like substance released by bradykinin (Fig. 1A versus 1B). Thus, the bradykinin-induced coronary vasodilation appears to be directly dependent on the endogenous generation of PGE-like substance.

DISCUSSION

There is little or no evidence of tissue storage of prostaglandins; thus, release is a reflection of *de novo* synthesis (15). Since prostaglandin synthetase is ubiquitous, synthesis of prostaglandins appears to be initiated by generation of the precursor, arachidonic acid, presumably by phospholipase activation (16). It was therefore surprising that bradykinin appeared to elicit a stoichiometric PGE-like substance release. However, the quantitative relationship between injected bradykinin and PGE-like substance in the coronary effluent may be misleading. The enhanced PGE-like substance release in the presence of SQ-20881 suggests that bradykinin is normally destroyed before or at the site of PGE-like substance synthesis. In fact, even with SQ-20881 present, only about 20% of the injected bradykinin was recovered in the coronary effluent, thus implying that considerably more than one molecule of PGE-like substance was released per molecule of bradykinin reacting. Furthermore, there was only a 50% recovery after injection of prostaglandin E_2 standards through the heart, compared to

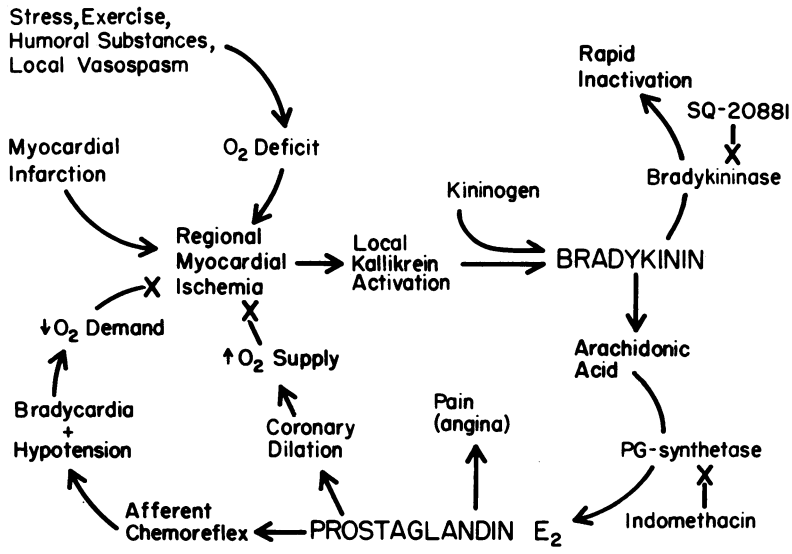

FIG. 2. Hypothetical scheme for the interrelationship between bradykinin and prostaglandin in the heart.

direct testing on the assay organs (not shown). These data, therefore, are consistent with a stimulation by bradykinin of catalytic generation of arachidonic acid. However, continuous cardiac infusion of bradykinin does not result in sustained PGE-like substance release or coronary dilation (not shown). This transient nature of the response to a continuing stimulus suggests the presence of negative feedback which curtails the receptor response; this could partially explain the limited but proportional cardiac response to bradykinin stimulation.

The observation that bradykinin-induced coronary vasodilation is mediated by endogenous prostaglandin biosynthesis fits with a number of recent reports concerning other actions of bradykinin. Thus, bradykinin has been demonstrated to cause prostaglandin release from the spleen (17, 18), lungs (19), kidney (20), uterus (21), blood vessels (22), and knee joint (23).

The possibility exists that PGE-like substance is actually an unstable endoperoxide intermediate, i.e., PGG_2 and/or PGH_2, instead of PGE_2 (24–26). These intermediates could be formed from hormone-released arachidonic acid in the myocardium and may even be responsible for the coronary vasodilation. The endoperoxides can be bioassayed because they contract rabbit aorta strips, whereas the final product PGE_2 is relatively inactive on this vascular smooth muscle (15). However, there was no evidence of a rabbit-aorta-contracting substance in the coronary venous effluent from hearts that were releasing PGE-like substance (stimulated by either bradykinin or exogenous arachidonic acid) (not shown). This does not rule out the possibility that the endoperoxides exert a biological effect and are rapidly transformed into PGE_2 and are not detected in the coronary effluent.

The demonstration that bradykinin is a potent stimulator of prostaglandin synthesis in the heart has important implications not only for normal cardiac function but especially for the activation of cardiovascular reflexes and pain in such cardiac disease states as myocardial infarction and angina pectoris. A working hypothesis which evolves from the current data and other published observations is presented in Fig. 2. This hypothesis states that various stimuli (endogenous or exogenous), or obstruction, cause an inappropriate cardiac oxygen demand relative to the oxygen supply; this (relative) regional ischemia activates kinin synthesis, which in turn stimulates local prostaglandin production, and this, alone or in combination with kinins, causes pain (angina) and coronary vasodilation and initiates afferent chemoreflexes. The bradykinin would function as a regulator of local blood flow (independent of nervous control), since its activity would be restricted largely to the site of formation by its rapid destruction (bradykininase). The induction of local kinin and prostaglandin synthesis would be expected to blunt part of the adverse effects of the myocardial ischemia, since the reflex bradycardia would decrease cardiac O_2 demand and the coronary dilation would increase the oxygen supply. Since prostaglandin would only be synthesized in the ischemic area of the heart, the coronary dilation would be restricted to the required site. The initiation of the pain sensation would serve as the signal of an inappropriate stimulus to the heart which must be withdrawn. Bradykinin, which has been found to have a strong pain-producing action, has been suggested as a substance responsible for the pain of angina pectoris (1).

To establish this hypothesis would require demonstration that: (*a*) kallikrein activation and kinin synthesis are quantitatively and temporally associated with myocardial ischemia; (*b*) kinin induces cardiac reflex and pain; (*c*) SQ-20881 (which decreases myocardial bradykinin destruction) enhances coronary dilation and reflex activation during myocardial ischemia; (*d*) coronary venous levels of prostaglandins (which directly reflect *de novo* cardiac synthesis) correlate with cardiac kinin levels; (*e*) there is simultaneous inhibition of prostaglandin synthesis (with indomethacin) and the bradycardia, hypotension, and pain produced by bradykinin or myocardial ischemia; (*f*) cardiac actions of bradykinin can be dissociated by means of synthetic analogs (for example analogs that do not cause prostaglandin synthesis should not activate pain or cardiovascular reflexes); and (*g*) specific antagonists of bradykinin receptor (not yet available) block both the prostaglandin synthesis and reflex activation caused either by exogenous bradykinin or myocardial ischemia.

A number of these criteria have already been fulfilled. Intracoronary injections of bradykinin have been demonstrated to cause a "coronary chemoreflex," i.e., hypotension

and bradycardia, which was also enhanced by a bradykininase inhibitor (27). Furthermore, there is some evidence supporting the local production of bradykinin in myocardial tissues (1, 27–29). There was a marked elevation of bradykinin in coronary sinus blood of dogs after occlusion of the anterior descending coronary artery (28). In addition, an activated kallikrein (i.e., kinin generating) system was observed in the coronary sinus and aortic blood in 7 of 11 patients with myocardial ischemia (29). Prostaglandins have been demonstrated to enhance the autonomic reflex response and pain produced by bradykinin in the spleen and the knee joint (17, 23). The rapid generation of prostaglandin by bradykinin in the heart (Fig. 1), could thereby generate the local chemical environment for modulating afferent sensory fibers. Finally, in recent experiments application of bradykinin to the left-ventricular wall of open-chested dogs caused excitation of afferent cardiac nerve fibers which was significantly reduced by pretreatment with aspirin (30). Aspirin-like drugs (e.g., indomethacin) are well known to inhibit prostaglandin synthesis (12) and might then be expected to abolish the prostaglandin facilitation of reflex responses from the heart, e.g., in myocardial infarction or angina pectoris.

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