Hormone Interactions in the Isolated Rabbit Heart
SYNTHESIS AND CORONARY VASOMOTOR EFFECTS OF PROSTAGLANDINS,
ANGIOTENSIN, AND BRADYKININ
By Philip Needleman, Garland R. Marshall, and Burton E. Sobel

ABSTRACT
In the present study, the synthesis and degradation of several potent vasoactive substances influencing coronary resistance were characterized in the isolated perfused rabbit heart. Prostaglandin synthetase activity, angiotensin converting enzyme activity, and bradykininase activity (without angiotensinase) were observed. A prostaglandin E2-like substance appeared to be the endogenous mediator of the coronary vasodilation produced by bradykinin and angiotensin II (All). (1) The concentration of this prostaglandin like substance in the coronary venous effluent was directly proportional to the concentration of the coronary vasodilator stimulus (bradykinin or All). (2) The prostaglandin-like substance released and the coronary dilation produced by the agonists correlated temporally and quantitatively. (3) Abolition of cardiac biosynthesis of the prostaglandin-like substance by indomethacin also abolished the decrease in coronary resistance produced by the agonists. All, the most potent naturally occurring vasoconstrictor substance, produced a paradoxical coronary vasodilation because it stimulated cardiac prostaglandin biosynthesis, but the direct coronary vasoconstrictor action of All could be readily unmasked by indomethacin, which blocks prostaglandin synthesis. The nonapeptide SQ-20881 blocked cardiac biosynthesis of All (from angiotensin I) and enhanced the coronary vascular effects of bradykinin by interfering with bradykininase activity. Similarly, the All-receptor antagonist, 1-Sar-8-Ile-AII, blocked the coronary vascular effects of All.

Since coronary resistance is influenced by neural, metabolic, and hormonal factors, the role of local vasoactive substances in the modulation of coronary blood flow has aroused considerable interest. One metabolite, adenosine, appears to mediate reactive hyperemia after coronary artery occlusion (1, 2), although the recent demonstration that prostaglandin E (PGE) biosynthesis occurs in the heart (3-6) suggests that this potent vasodilator material also participates in local regulation of myocardial blood flow. Locally synthesized PGE may affect the coronary vasculature not only directly but also by influencing afferent and efferent neural activity impinging on vasomotor tone (7, 8). Moreover, other vasoactive substances including bradykinin may exert important humoral influences on coronary resistance both by acting directly on resistance vessels and by inducing prostaglandin release (9). Angiotensin, one of the most powerful direct-acting coronary vasoconstrictor substances known, can influence myocardial perfusion indirectly by enhancing postsynaptic norepinephrine release from stimulated adrenergic nerves (10) and by augmenting myocardial contractility and oxygen requirements. In view of the potential importance of the effects of these vasoactive substances on coronary resistance, the present investigation was undertaken to assess cardiac prostaglandin biosynthesis and evaluate several local interrelationships between prostaglandins, angiotensin, and bradykinin that may affect myocardial perfusion under physiological and pathological conditions.

Methods
PERFUSED RABBIT HEART PREPARATIONS
Male New Zealand rabbits weighing 1.5-2 kg were anesthetized with sodium pentobarbital (30 mg/kg, iv) and given heparin (100 units/kg, iv). Their hearts were removed and perfused retrograde through an aortic cannula with oxygenated (95% O2, 5% CO2) Krebs-Henseleit solution (37°C) at a constant flow of 30 ml/min. A Statham pressure transducer was utilized to monitor perfusion pressure at the level of the aortic valve (Brush recorder, Gould Instrument Co.).
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SUPERFUSED ORGAN SYSTEMS USED FOR ASSAY OF VASOACTIVE SUBSTANCES

The effluent (30 ml/min) from the isolated perfused rabbit hearts was continuously and immediately monitored for the presence of vasoactive substances by superfusing a series of assay tissues with the coronary outflow according to the procedure of Vane (11, 12). Assay tissues included the rat stomach strip and the chick rectum, both particularly sensitive to prostaglandins, especially of the E type, rat colon strips, sensitive to rabbit hearts was continuously and immediately monitored for the presence of vasoactive substances by superfusing a series of assay tissues with the coronary outflow according to the procedure of Vane (11, 12). Assay tissues included the rat stomach strip and the chick rectum, both particularly sensitive to prostaglandins, especially of the E type, rat colon strips, sensitive to prostaglandin F2α, and angiotensin II (All) but insensitive to bradykinin but not to angiotensin or prostaglandins (11, 12). Selected antagonists were added to the coronary effluent to render the superfusion assay tissues insensitive to catecholamines, acetylcholine, serotonin, and histamine (11, 12). The sensitivity of the assay organs to prostaglandins was enhanced with indomethacin (to block endogenous prostaglandin synthesis in the assay tissues themselves) perfused at 10 μg/min across the assay organs but distal to the heart (13). The assay organs were calibrated with prostaglandin standards at the beginning and the conclusion of each experiment.

VERIFICATION OF RESULTS OBTAINED WITH THE BIOASSAY PROCEDURES

Several substances detected in the coronary effluent in these studies appeared to be prostaglandins, judging from the response and the characteristics of the systems used for bioassay. Disappearance of activity after treatment with indomethacin, a specific inhibitor of prostaglandin biosynthesis (14), corroborated this impression. Further elucidation of the nature of the prostaglandin-like materials released from the heart was obtained by extracting coronary sinus effluent with ethyl acetate after adjustment to pH 3 with formic acid, evaporating the solvent, and chromatographing the extract on silica gel G plates in a chloroform-methanol-acetic acid solution (95:5:5). The migration of the vasoactive material was compared with that of prostaglandin standards in the same chromatographic system. Furthermore, elution of material from the plate at spots corresponding to the locus of a PGE standard provided the recovery of a material that contracted the rat stomach and the chick rectum (i.e., the eluted material behaved identically to PGE in the bioassay system).

MATERIALS

The prostaglandin standards were kindly supplied by the Upjohn Company, the indomethacin by Merck, Sharp and Dohme, and the SQ-20881 by the Squibb Company. SQ-20881 is a nonapeptide originally isolated from snake venom which blocks the pulmonary conversion of AI to All and the pulmonary destruction of bradykinin (15).

Results

CARDIAC SYNTHESIS AND CLEARANCE OF ANGIOTENSIN

Rat colon strips were employed as superfusion assay organs for the detection and quantification of All in the coronary venous effluent. The decapeptide AI was less potent in contracting this assay organ (which itself lacks converting enzyme activity) when it was injected directly across the rat colon than it was when it was injected proximal to the perfused rabbit heart (Fig. 1). The enhancement of the rat colon response to AI by passage of the peptide through the heart suggests rapid conversion of the decapeptide to the octapeptide All. In contrast, All was more potent when it was tested directly on the rat colon than it was after circulation through the heart. Continuous infusion of the heart with the nonapeptide SQ-20881 (previously reported to be an inhibitor of pulmonary AI-to-All converting enzyme) reversed the response produced by AI. Under these conditions, AI was more potent when it was applied directly across the assay organ than it was when it was administered in the coronary perfusate. SQ-20881 did not significantly alter the direct response of rat colon strips to Al (data not shown) nor did it change the responses of the rat colon to All applied directly or after passage through the coronary circulation. However, the All receptor inhibitor 1-Sar-8-Ile-AlI (at 2 μg/min) completely blocked the AI- and the All-induced contractions of the rat colon.

All was 25 times more potent than its precursor Al when it was tested directly on rat colon strips (Fig. 1). In contrast, after passage through the coronary circulation All exhibited a 50% loss of activity detectable by bioassay in contrast to AI which exhibited a twofold increase (Fig. 1). Treatment of the heart with SQ-20881 to block apparent
cardiac converting enzyme activity led to a 50% decrease in contraction of the bioassay organ exposed to coronary venous effluent when AI was added to the coronary perfusate.

Thus, the perfused rabbit heart possesses substantial converting enzyme activity, since in one passage across the heart approximately 20% of the administered AI is converted to AII. As is the case with the pulmonary converting enzyme, SQ-20881 appears to effectively block cardiac biosynthesis of AII from AI. However, unlike the case for several other tissues such as the kidney, the heart contains little angiotensinase activity.

**ANGIOTENSIN II-INDUCED CARDIAC PROSTAGLANDIN SYNTHESIS**

When AII was presented through the coronary circulation within 15 minutes after initiation of perfusion, coronary resistance increased and PGE-sensitive assay organs revealed no release of a prostaglandinlike substance (Fig. 2). The heart did have the capacity to synthesize prostaglandinlike substances at this time, however, as indicated by experiments with bradykinin. When the AII infusion was repeated 1–2 hours after the initiation of perfusion (late infusion) of the isolated rabbit heart, a concentration-dependent coronary vasoconstriction associated with a pronounced simultaneous release of a prostaglandinlike substance was observed (Fig. 2). The AII receptor blocker 1-Sar-8-Ile-AII (infused through the heart at 100 ng/ml min⁻¹) completely abolished the coronary resistance and the prostaglandinlike substance stimulation produced by these relatively late infusions of AII (data not shown).

Treatment of the heart with indomethacin (either immediately after or 2 hours after initiation of perfusion) blocked the release of the prostaglandinlike substance and the coronary dilation induced by AII (Fig. 2 and Table 1). In the absence of the production of the prostaglandinlike substance either during early infusions or when indomethacin was administered, the release of a prostaglandinlike substance during AII infusions was blocked.

**TABLE 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>15 ng AII/ml (N = 4)</th>
<th>30 ng AII/ml (N = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary pressure change (mm Hg)</td>
<td>+6 ± 4</td>
<td>+16 ± 3</td>
</tr>
<tr>
<td>PLS release (ng)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>After indomethacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary pressure change (mm Hg)</td>
<td>−8 ± 3</td>
<td>−17 ± 3</td>
</tr>
<tr>
<td>PLS release (ng)</td>
<td>16 ± 3</td>
<td>54 ± 8</td>
</tr>
<tr>
<td>Coronary pressure change (mm Hg)</td>
<td>0</td>
<td>+14 ± 5</td>
</tr>
<tr>
<td>PLS release (ng)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Coronary pressure was measured during a constant-flow (30 ml/min) infusion. Prostaglandinlike substance (PLS) was calculated by measuring the contractile response of the chick rectum produced by the AII infusion in comparison with the response produced by infusion of different concentrations of PGE₂ standards. All values are means ± se, and the number of rabbits tested is given in parentheses.
methacin accompanied late infusions, All produced only coronary vasoconstriction.

**BRADYKININ-INDUCED CORONARY VASODILATION AND SYNTHESIS OF A PROSTAGLANDINLIKE SUBSTANCE**

Exogenous bradykinin produced a decrease in coronary resistance associated with the simultaneous appearance of a prostaglandinlike substance in the coronary venous effluent (Figs. 2-4). Both the coronary dilation and the release of the prostaglandinlike substance were enhanced by SQ-20881 and completely inhibited by indomethacin (Figs. 3 and 4). In addition, indomethacin pretreatment of the heart completely abolished the release of the prostaglandinlike substance by bradykinin (Figs. 3 and 4).

Effects of the prostaglandinlike substance released by selected doses of exogenous bradykinin were compared with the effects of PGE$_2$ standards applied directly across the same assay organs (Fig. 4 shows the chick rectum response; comparable data were obtained on the rat stomach strip). The release of the prostaglandinlike substance from the heart in response to bradykinin paralleled the effects of PGE$_2$ standards in a dose-dependent fashion. The prostaglandinlike material appeared to be PGE$_2$, judging from thin-layer chromatography and the inhibition of its biosynthesis that followed treatment with indomethacin.

Since the cat jejunum responds to bradykinin but not to prostaglandins or All, this assay organ was used to determine whether the rabbit heart inactivates bradykinin. Dose-response comparisons for the presence of bradykinin in the coronary effluent were performed. Direct application of 9 ± 1 ng (N = 6) of bradykinin produced a 2-cm contraction; when bradykinin was injected through the heart, 85 ± 7 ng (N = 6) of bradykinin was required to produce a comparable contraction. Thus, 90% of the bradykinin was lost in one passage across the heart. However, when bradykinin was injected into the coronary circulation of a heart treated with SQ-20881 (160 ng/ml, a known bradykininase inhibitor), then only 24 ± 4 ng (N = 5) was necessary to produce a 2-cm contraction of the cat jejunum. The reduction of bradykinin inactivation was associated with both increased coronary dilation and enhanced release of the prostaglandinlike substance.

Sustained coronary infusion of bradykinin produced sustained contraction of the bradykinin-sensitive assay organ (cat jejunum) with only transient release of a prostaglandinlike substance from the heart, evidenced by the brief contraction of the prostaglandin-sensitive chick rectum and rat stomach. Transient coronary dilation was associated with transient release of the prostaglandinlike substance (Fig. 5). In contrast, infusion of arachidonic acid, a precursor of PGE$_2$, caused sustained coronary dilation with sustained release of the prostaglandinlike substance. The sustained cardiac synthesis of the prostaglandinlike substance
and the sustained coronary dilation produced by arachidonic acid did not modify (inhibit or enhance) the transient cardiac response to a simultaneous sustained infusion of bradykinin (Fig. 5). Indomethacin immediately inhibited the cardiac synthesis and release of the prostaglandinlike substance induced by arachidonic acid or bradykinin (not shown). Thus, in response to both arachidonic acid and bradykinin, coronary vasodilation appears to reflect effects of a prostaglandinlike substance on the vasculature.

Rabbit aorta strips were included in the assay system for the detection of any rabbit aorta-contracting substance (prostaglandin endoperoxide) in the coronary effluent (16). No rabbit aorta-contracting substance was detected in any experiments even at times of maximum release of the prostaglandinlike substance resulting from cardiac infusions of either arachidonic acid or bradykinin (not shown).

Discussion

INTERACTIONS BETWEEN ANGIOTENSIN, BRADYKININ, AND PROSTAGLANDINLIKE SUBSTANCE

Low but detectable levels of AI converting enzyme activity occur in the rat heart (17), and the increased coronary resistance produced by AI is blocked by antibody to AI or by converting enzyme inhibitor (18, 19). Our results indicate that, as in the lung, AI converting enzyme activity in the heart is coupled to bradykininase activity. Thus, the nonapeptide SQ-20881 simultaneously enhances the myocardial bradykinin response and blocks the activation of AI (Figs. 1-4). The relative lack of angiotensinase activity (Fig. 1) and the presence of converting enzyme (and hence bradykininase) activity suggest that in the heart effects of bradykinin can be exerted only transiently in restricted local regions, whereas it appears that local myocardial formation of AI from conversion of AI can produce relatively persistent effects.

AI exerts several well-recognized effects on the heart. It augments the release of norepinephrine produced by adrenergic nerve stimulation (10, 20, 21) and it increases contractility (22, 23). These properties suggest that AI increases cardiac work and oxygen demand. In addition, AI can decrease oxygen delivery by direct coronary artery constriction. The present findings suggest an endogenous mechanism that blunts potentially detrimental effects of AI. Thus, the prostaglandinlike substance released in association with humoral, metabolic, or neural stimulation may suppress AI-induced norepinephrine release, thus reducing myocardial oxygen demand, and dilate coronary vessels directly, possibly increasing oxygen supply.

Unlike bradykinin, AI does not cause biosynthesis and release of a prostaglandinlike substance early during perfusion of the isolated heart (Fig. 2). However, after 1-2 hours of perfusion, when the basal release rate of the prostaglandinlike substance has generally increased, AI produces a coronary vasodilation that is associated with the release of a prostaglandinlike substance and reversible by indomethacin administration (Fig. 2, Table 1). Analogous results have been obtained in isolated perfused spleen or kidney preparations (24, 25). Terragno et al. (26) have shown in pregnant dogs that AI actually increases uterine blood flow, an effect which is reversed following treatment with indomethacin. The potential importance of endogenous prostaglandin synthesis in regulating myocardial blood flow is exemplified by the fact that the most potent vasoconstrictor substance in the body, AI, produces coronary dilation when it results in the release of a prostaglandinlike substance by the heart (Fig. 2). The concentrations of AI required to induce such a release are high compared with the circulating levels appearing during pathological conditions. However, since the heart can synthesize AI by conversion from AI, high local concentrations could possibly be achieved. The concentration of the prostaglandinlike substance generated at the coronary vascular contractile site must be very high, since it blunts the direct effects of AI.

In the present study, bradykinin produced a concentration-dependent decrease in coronary resistance that was directly associated with the biosynthesis and release of a prostaglandinlike substance.
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substance; this effect was abolished by indomethacin and enhanced by SQ-20881 (Figs. 3 and 4). Since the isolated perfused rabbit heart can immediately convert arachidonic acid to a prostaglandinlike substance and since prostaglandins are generally not stored in tissues, bradykinin probably stimulates prostaglandin synthesis by providing arachidonate. Generation of this prostaglandin precursor from membrane phospholipid by bradykinin may result from activation of phospholipase A2 (27). However, provision of arachidonate by bradykinin may be a self-limiting process, perhaps reflecting feedback inhibition. Chronic infusion of bradykinin results in transient early release of a prostaglandinlike substance in contrast to the sustained release of such a substance associated with infusion of arachidonate directly (Fig. 5). Thus, the limiting step must be at some point preceding the prostaglandinlike substance synthetase step. Another possibility is that arachidonate causes feedback inhibition of the phospholipase A2. This situation is apparently not the case, since bradykinin infusion produces the same response without inhibition or enhancement in the presence of a continuous arachidonic acid infusion (Fig. 5).

Coronary dilation produced by bradykinin appears to be mediated by endogenous coronary biosynthesis of a prostaglandinlike substance (9). This conclusion is supported by the temporal correlation between the changes in coronary resistance and the appearance of the prostaglandinlike substance in the coronary venous effluent (Fig. 5) and by the complete inhibition of bradykinin-induced coronary vasodilation by treatment with indomethacin (or aspirin or meclofenamate, both of which also inhibit prostaglandin biosynthesis).

Bradykinin does not generally persist in arterial blood because of an efficient destruction mechanism in the lung. Thus, kinins could influence coronary blood flow markedly only if cardiac bradykinin synthesis occurs. Bradykinin is elevated appreciably in the coronary sinus blood of dogs after occlusion of the anterior descending coronary artery (28). Furthermore, an activated kallikrein (i.e., kinin-generating) system has been observed in coronary sinus and aortic blood in 7 of 11 patients with myocardial ischemia (29).

Results in the present study indicate that the heart possesses systems capable of inactivating bradykininase rapidly. Cardiac bradykininase activity is inhibited by SQ-20881 (Figs. 3 and 4). Identification of both kinin synthesis and inactivation systems in the heart is consistent with the possibility that bradykinin plays a local hormonal role in this organ.

MYOCARDIAL HORMONE INTERACTIONS

The isolated rabbit heart has the capacity to synthesize, release, and degrade potent vasoactive substances which can readily modulate coronary resistance (Fig. 6). Data obtained with an isolated Krebs-perfused rabbit heart are only suggestive and necessitate confirmation in intact animals. The current experiments demonstrate the presence of a prostaglandinlike substance synthetase (arachidonate to prostaglandinlike substance), converting enzyme (AII biosynthesis from A1), and bradykininase (enzymatic destruction of bradykinin) in the heart. The interactions between and the pharmacological manipulation of these endogenous pathways can radically alter coronary vascular responsivity. Thus, the decrease in coronary resistance produced by arachidonic acid, bradykinin, or AII is mediated by the synthesis and release of a prostaglandinlike substance and is blocked by indomethacin. The nonapeptide SQ-20881 exerts two effects in the heart: (1) it prevents the activation of angiotensin by inhibiting myocardial converting enzyme, and (2) it enhances the effect of bradykinin by interfering with myocardial bradykininase. Of course, SQ-20881 would not be expected to inhibit AII already present in arterial blood (synthesized in the lung). However, the octapeptide analogue 1-Sar-8-Ile-AII is a potent specific inhibitor of AII in the heart. The prostaglandinlike substance appears to mediate some responses of the coronary vasculature to pathological conditions such as anoxia, high-frequency neural stimulation,

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FIGURE 6

Interactions of humoral substances in the heart. AI = angiotensin I, AII = angiotensin II, BK = bradykinin, PGE2 = prostaglandin E2, arrow = stimulation, and X = inhibition.
and high blood levels of vasoactive substances. Under these conditions, the prostaglandinlike sub-
stance may act to improve the balance between cardiac oxygen supply and demand (Fig. 6) by
producing (1) a decrease in coronary resistance, (2) activation of afferent chemoreflexes leading
to bradycardia and hypotension (30-32), and (3) suppression of efferent adrenergic stimulation (33-35).

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