Quantitative pharmacologic responses of normal and atherosclerotic isolated human epicardial coronary arteries

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ABSTRACT We studied quantitative aspects of coronary artery contraction in isolated epicardial coronary ring segments from 49 human hearts. The order of maximal tension developed by drugs in normal calcium (ionized calcium, 1.26 mM) solution was U-44069 (a prostaglandin endoperoxide analog) > histamine > carbachol > serotonin > phenylephrine > ergonovine. In Ca\(^{2+}\)-free solution these same drugs mediated a lesser degree of contraction, which demonstrates that the human coronary artery uses both "intracellular" and "extracellular" calcium in hormone receptor-activated contraction. U-44069, histamine, carbachol, and phenylephrine produced calcium-free/normal calcium maximal responses of 62.9%, 48.7%, 39.8%, and 37.2%, respectively. Morphologic characteristics of the atherosclerotic plaques within the vessel lumen and the degree of myocardial dysfunction did not qualitatively alter these contractile responses. However, severely atherosclerotic coronary segments were supersensitive to histamine, but not to carbachol or calcium. In conclusion, the human epicardial coronary artery is a highly reactive vessel that uses at least two calcium pools to couple contraction. Receptor-coupled agonists differ in their abilities to mediate contraction and in the degree to which each calcium pool is used, and the presence of atherosclerosis potentiates the contractile response to histamine.


VASOMOTION and altered pharmacologic reactivity of the human epicardial coronary artery may play an important role in pathophysiologic mechanisms involved in ischemic heart disease.\(^1\) Coronary arterial spasm, an abnormal exaggerated contraction of the smooth muscle of the epicardial coronary artery, is now recognized as a potential cause of unstable, variant, and typical effort angina pectoris, as well as acute myocardial infarction.\(^2\)

Babour\(^3\) was the first to report that the isolated human epicardial coronary artery responds pharmacologically to vasoactive stimuli. In 1912 he demonstrated that cadaveric coronary ring segments contract when exposed to epinephrine. More recently, other investigators have described qualitative pharmacologic responses of the isolated human coronary artery to a variety of vasoactive drugs, but limited availability of tissue has precluded extensive quantitative analysis.\(^4-8\)

In this investigation, we have examined large quantities of isolated human epicardial coronary arteries that were freshly procured. The pharmacologic responses to histamine, phenylephrine, serotonin, ergonovine, carbachol, and the prostaglandin endoperoxide analog U-44069 have been characterized, and the calcium pools mediating their contractile effects have been identified. Additionally, the effect of atherosclerosis on the contractile response caused by several vasoactive drugs has been evaluated. The results indicate receptor-type specific utilization of multiple calcium pools in coupling contraction and also indicate that the presence of atherosclerosis potentiates the contractile response of at least one drug capable of provoking coronary spasm.

Methods

Patient characteristics. Coronary arteries were obtained from 49 human hearts. Four of the 49 hearts (group A) were normally functioning donor hearts that were not used for transplantation because of medical complications in the awaiting recipient. Group B consisted of 20 hearts with end-stage idiopathic congestive cardiomyopathy. Severe coronary artery dis-
ease with resulting myopathic ventricles ("ischemic cardiomyopathy") was present in 17 hearts (group C). Six hearts (group D) were transplanted hearts removed because of severe coronary disease, and two hearts (group E) were from patients with primary pulmonary hypertension who underwent combined heart-lung transplantation. Table 1A lists the mean age and length of clinical symptoms for each group of these patients.

For morphologic analysis of the coronary arteries and correlation of morphologic characteristics with pharmacologic function, 31 of the 49 hearts were examined histologically. Of these 31 hearts, two were from group A, 15 from group B, eight from group C, four from group D, and two from Group E.

None of the 49 patients were on adrenergic, muscarinic, histaminic, or calcium receptor-blocking drugs within 2 weeks of study.

**Tissue bath studies.** Upon excision and removal, the hearts were immediately placed in ice-cold, oxygenated, physiologic salt solution, and the left anterior descending artery, circumflex artery, and right coronary artery were dissected free. Each coronary artery was cleaned of all adherent fat and connective tissue and was sectioned into 5 mm ring segments. Attention and care were given to maintaining and not disrupting the integrity of the endothelium. The only segments discarded were those that were completely occluded, through which a mounting pin could not be placed. The time from cardiectomy to placement in the muscle bath was less than 35 min.

The coronary ring segments were placed in a multichamber tissue bath. Ring segments were attached by two metal pins (0.018 inches) and were then attached to a Gould (UC-3) force-displacement transducer. Normal physiologic solution (modified Tyrode's) consisted of the following composition in mM: NaCl, 118; KCl, 4; MgSO4, 1.2; CaCl2, 2; dextrose, 5; NaHCO3, 24; NaH2PO4, 1.2. The ionized calcium was 1.26 mM as measured by an ion-specific electrode (AMT Clin-Ion, Applied Medical Technology; Palo Alto, CA). Calcium-free solution was similarly composed except for the omission of calcium and the addition of 0.5 mM EGTA. In this solution no Ca2+ could be detected by the ion-specific electrode. The pH was kept constant at 7.40 to 7.45, and each bath was aerated with 95% O2 and 5% CO2. The ring segments were equilibrated for 60 to 90 min. Approximately 1.2 g of resting tension was applied to each ring segment. Previous studies in our laboratory have determined that this is the optimal tension for obtaining a maximal contractile response to the agonists studied. Recordings were made on a multichannel physiologic recorder with light-sensitive paper.

**Histologic examination.** Histologic examination was performed on all coronary ring segments from 31 of the 49 hearts, a total of 523 segments. After measurement of pharmacologic response, the segments were fixed in 10% formalin and stained with hematoxylin and eosin. The coronary artery segments were coded, examined, and scored by two observers in a semiquantitative fashion.

Four morphologic characteristics were used for scoring purposes: (1) degree of luminal occlusion, (2) degree of fragmentation of internal elastic lamina, (3) degree and distribution of calcium, and (4) degree of intimal proliferation. Each individual category was scored on a scale from 1 to 3, with 1 being normal and 3 being severely diseased. A total coronary artery disease score (CADS) was generated for each segment, and this value is a mean of the four individual morphologic characteristics (figure 1). Mean values from two independent readings comprised the final score. Table 1B gives the CADS according to decade of the heart from which the vessels were obtained.

The histologic sections of the coronary segments were also classified according to the specific shape of the atherosclerotic plaque present within the lumen. The vessels either had concentric lesions (atheromatous plaque uniformly distributed around the inner circumference of vessel) or eccentric lesions (atheromatous plaque occupying 50% or less of the inner circumference). Maximal grams tension was then examined according to these two morphologic characteristics.

**Pharmacologic techniques.** All drugs were added to the bath in a cumulative fashion in 0.3 or 0.5 log unit increments. Full log dose-response curves to each agonist were performed in each coronary ring segment. The only exception to this was with U-44069, since the maximal concentration of drug available to us was 10-9 M. A full dose-response curve was determined by demonstrating a maximal response, which was defined as no further increase in contraction with subsequent administration of a higher dose of agonist. Normalized dose-response curves were then constructed on a percent of maximum basis, with 0% equaling resting tension and 100% equaling maximal response. The ED50 is defined as the concentration of agonist at which 50% of the maximal response is obtained. Previous studies in our laboratory demonstrated reproducibility of responses for at least 10 hr, much longer than the time needed to complete these specific studies. The drugs were administered in random order for each heart studied, and this procedure helped to minimize the possibility that time in the organ bath was a factor when the tissue was exposed to an agonist or that previous exposure to another agonist interfered with the subsequent pharmacologic response of the agonist. For histamine, the effects of cimetidine, an H2-receptor–blocking agent that prevents any receptor activated – smooth muscle relaxation,9 were initially evaluated in six hearts. Cimetidine at 3 × 10-5 M had no effect on the dose-response curve of histamine for muscle contraction and was not routinely used in subsequent experiments. Calcium dose-response curves were generated by the cumulative addition of calcium to potassium-depolarized segments, and the ionized calcium concentration was determined with the ion-specific electrode at each measurement taken.

Total tension was recorded as the absolute tension minus the baseline or resting tension. Although the ring segments were all of uniform length (5 mm), the diameters of the vessels varied. To compensate for any difference in the size of the media in the different segments might have had in the total tension generated, the area of the media relative to total vessel area was determined with planimetry by a Summa Graphics Bit Pad connected to a Hewlett-Packard 1000 computer. To measure the effects of atherosclerosis (CADS) on contractile function, total tension was expressed as maximal milligrams of total tension development and as milligrams tension per square millimeter of media.

The following drugs were used in this study: histamine dichloride, carbachol, phenylephrine, 5-hydroxytryptamine (5-HT), ergonovine maleate, atropine, and calcium chloride (all from Sigma Chemical Co., St. Louis). The α1-agonists, prazosin (Pfizer Laboratories, New York), the 5-HT1 antagonist, ketanserin (Janssen Laboratories, New Brunswick, NJ),...
and the H₂ antagonist, pyrrobutamine (Smith, Kline & French Research Ltd., Welwyn, England), were also used. U-44069, a stable PGH₂ endoperoxide, was obtained from Upjohn Co. (Kalamazoo, MI), courtesy of Dr. John Pike.

Statistics. Significance of data was analyzed by Student’s two-tailed t test with unpaired means and the Bonferroni multiple comparison test. A p value < .05 was considered statistically significant.

Results

Calcium pools and maximal contractile response. The maximal contractile responses in normal physiologic salt solution (expressed in milligrams of tension generated above resting tension) are presented in table 2. Coronary ring segments were used in this analysis regardless of the extent of their disease, of regional location, or of the vessel examined. Forty percent of the segments exposed to phenylephrine and 35% exposed to ergonovine did not respond, although these same segments responded when exposed to U-44069, histamine, carbachol, or calcium. All segments that did respond contracted and did not relax in the presence of the agonists used. In a normal calcium solution, U-44069 generated the greatest grams tension of all the agonists examined. The rank order of maximum grams tension generated by these vasoactive drugs operating through a receptor-operated pathway was: U-44069 > histamine > carbachol > serotonin > phenylephrine > ergonovine. Depolarization of the vessel with 40 mM KCI generated 660 ± 39 mg tension. The contractile response to 10⁻⁵M carbachol was blocked by 10⁻⁷M atropine, but not by 10⁻⁸M prazosin or 10⁻⁷M pyrrobutamine; the response to 10⁻⁷M histamine was blocked by 10⁻⁷M pyrrobutamine, but not by 10⁻⁷M atropine or 10⁻⁸M prazosin; the response to 10⁻⁶M phenylephrine was blocked by 10⁻⁷M prazosin, but not by 10⁻⁵M atropine or 10⁻⁷M pyrrobutamine; the response to 10⁻⁵M serotonin was antagonized by ketanserin in concentrations from 10⁻⁹M to 10⁻⁷M.¹¹

Figures 2A and 2B demonstrate the two distinct sources of calcium the human coronary artery uses to mediate contraction.¹² In normal calcium solution (figure 2A) the maximal response to an agonist, in this case carbachol, is sustained. However, in a calcium-free solution (figure 2B), the same agonist generates a
TABLE 2
Maximal contractile response (mean ± SE)

<table>
<thead>
<tr>
<th>Maximal response in normal calcium (1.26 mM Ca²⁺) (mg)</th>
<th>Maximal response in calcium-free solution (mg)</th>
<th>Milligrams tension Ca²⁺ free/normal Ca²⁺ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-44069 (10⁻⁶M)</td>
<td>1510 ± 34 (190)^a</td>
<td>950 ± 63 (85)</td>
</tr>
<tr>
<td>Histamine (10⁻⁴M)</td>
<td>1270 ± 91 (476)</td>
<td>619 ± 91</td>
</tr>
<tr>
<td>Carbachol (10⁻⁵M)</td>
<td>1080 ± 45 (386)</td>
<td>430 ± 59 (47)</td>
</tr>
<tr>
<td>Serotonin (10⁻⁵M)</td>
<td>768 ± 112 (66)</td>
<td></td>
</tr>
<tr>
<td>KCl (40 mM)</td>
<td>660 ± 39 (185)</td>
<td></td>
</tr>
<tr>
<td>Phenylephrine (10⁻⁵M)</td>
<td>600 ± 75 (224)</td>
<td>223 ± 113 (21)</td>
</tr>
<tr>
<td>Ergonovine (10⁻⁵M)</td>
<td>540 ± 138 (132)</td>
<td></td>
</tr>
</tbody>
</table>

^aNumerals in parentheses indicate number of segments.
^bStatistically different (p < .05) from U-44069.
^cStatistically different (p < .05) from U-44069.

contraction that is not sustained and is of a lesser degree of tension compared with that in normal calcium solution.

Table 2 also gives the maximal contractile response to histamine, carbachol, and U-44069 in a calcium-free physiologic salt solution. The rank order of milligrams tension caused by drugs is similar to that in normal calcium solution (U-44069 > histamine > carbachol). The data are also presented as a percentage of the milligrams tension generated in a calcium-free solution compared with that in normal calcium solution. In calcium-free solution, as determined by the ion-specific electrode, U-44069 produces a relatively greater percent contractile response than histamine (p < .05), carbachol (p < .05), or phenylephrine (p < .05).

Histologic analysis and maximal contractile response.

Table 1A includes the calculated CADS for the patients according to their category of clinical group. The score is a total mean value (see Methods) and ranges from 1.00 (normal) to 3.00 (severely diseased). Examination of the data reveals that even in the "normal" hearts from 21-year-old subjects (group A), there is some atherosclerotic disease present. In the patients with a congestive myopathy but no history of coronary disease (group B), there was slightly more

FIGURE 2A. Contractile response of six coronary ring segments to carbachol (10⁻⁵M). The response is sustained over time (10 min) once maximal tension is achieved. The maximal tension obtained by each segment varies according to underlying degree of atherosclerosis. The small undulations in the tracings are electronic noise detected by the recorder from other laboratory equipment.
TABLE 3

Human coronary artery maximal response according to extent of atherosclerosis (n = 31 hearts, mean ± SE)

<table>
<thead>
<tr>
<th>CADS</th>
<th>1.00</th>
<th>1.13</th>
<th>1.25</th>
<th>1.38</th>
<th>1.50</th>
<th>1.63</th>
<th>1.75</th>
<th>1.88</th>
<th>2.00</th>
<th>2.13</th>
<th>2.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine (mg)</td>
<td>1560</td>
<td>960</td>
<td>1160</td>
<td>1100</td>
<td>1350</td>
<td>1080</td>
<td>1240</td>
<td>1160</td>
<td>1120</td>
<td>730</td>
<td>1170</td>
</tr>
<tr>
<td>± 340</td>
<td>± 280</td>
<td>± 180</td>
<td>± 190</td>
<td>± 210</td>
<td>± 220</td>
<td>± 160</td>
<td>± 120</td>
<td>± 160</td>
<td>± 230</td>
<td>± 170</td>
<td></td>
</tr>
<tr>
<td>Phenylephrine (mg)</td>
<td>320</td>
<td>430</td>
<td>420</td>
<td>350</td>
<td>690</td>
<td>230</td>
<td>690</td>
<td>420</td>
<td>780</td>
<td>460</td>
<td>470</td>
</tr>
<tr>
<td>± 70</td>
<td>± 110</td>
<td>± 60</td>
<td>± 90</td>
<td>± 90</td>
<td>± 80</td>
<td>± 160</td>
<td>± 120</td>
<td>± 160</td>
<td>± 180</td>
<td>± 110</td>
<td></td>
</tr>
<tr>
<td>Carbachol (mg)</td>
<td>1870</td>
<td>1640</td>
<td>1780</td>
<td>1280</td>
<td>1440</td>
<td>1240</td>
<td>1290</td>
<td>1350</td>
<td>920</td>
<td>720</td>
<td>830</td>
</tr>
<tr>
<td>± 300</td>
<td>± 230</td>
<td>± 280</td>
<td>± 230</td>
<td>± 30</td>
<td>± 760</td>
<td>± 160</td>
<td>± 220</td>
<td>± 110</td>
<td>± 200</td>
<td>± 150</td>
<td></td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1730</td>
<td>1990</td>
<td>1720</td>
<td>1410</td>
<td>1830</td>
<td>1940</td>
<td>1500</td>
<td>1370</td>
<td>1440</td>
<td>1490</td>
<td></td>
</tr>
<tr>
<td>± 160</td>
<td>± 280</td>
<td>± 260</td>
<td>± 220</td>
<td>± 180</td>
<td>± 220</td>
<td>± 340</td>
<td>± 200</td>
<td>± 200</td>
<td>± 280</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ergonovine (mg)</td>
<td>410</td>
<td>330</td>
<td>120</td>
<td>230</td>
<td>490</td>
<td>110</td>
<td>490</td>
<td>310</td>
<td>190</td>
<td>300</td>
<td>280</td>
</tr>
<tr>
<td>± 60</td>
<td>± 100</td>
<td>± 50</td>
<td>± 110</td>
<td>± 180</td>
<td>± 20</td>
<td>± 140</td>
<td>± 110</td>
<td>± 50</td>
<td>± 140</td>
<td>± 90</td>
<td></td>
</tr>
</tbody>
</table>

*Values to the right of this point are statistically different (p < .05) from tension generated at CADS = 1.00.

Atherosclerosis present than in group A, which probably reflects the older mean age of group B. Group C patients with known coronary disease had a mean score of 2.28, and this represents moderate-to-severe atherosclerotic involvement.

Table 1B gives the data for CADS by decade with all clinical categories grouped together. Subjects in the second and third decade of life have minimal but definite coronary atherosclerosis. The degenerative disease process then rapidly progresses in severity in the fourth and fifth decades of life.

The maximal tension generated by each agonist relative to the calculated CADS is given in table 3. The response to calcium was uniform until a CADS score of 2.63 was reached, after which maximum tension declined. The maximum grams tension generated by receptor-mediated agonists also decreased when advanced atherosclerosis was present. However, the response to carbachol appeared to be affected by relatively milder degrees of atherosclerotic change, as maximal tension declined near the midpoint of the CADS.

FIGURE 2B. Response to carbachol (10⁻⁵M) in a calcium-free solution. The six coronary ring segments (different group from figure 2A) generate overall less contraction than that in a normal calcium solution, and the response is not sustained.
The data for carbachol and histamine were also normalized to the maximum response produced by calcium, a data transformation that should correct for differences in amount of contractile unit. The disease-related decrement in the maximum response to these agonists persists after normalization.

Table 4A gives the relationship of media to total vessel area. Although the diameter of the epicardial coronary artery decreased distally, the ratio of the area of the media to the total vessel area of any segment remained relatively uniform. As shown in table 4B, there was a decrease in tension per square millimeter with a higher CADS. This decrease in tension with increasing atherosclerosis occurred whether the tension was corrected for media area as in table 4B or whether it was normalized to calcium.

The CADS according to location of the coronary segment within the coronary artery is presented in table 5. For the purpose of analysis, the right coronary artery, left anterior descending artery, and circumflex artery were divided into proximal, middle, and distal portions by conventional angiographic criteria. Groups A, B, C, and D were analyzed in the same manner. In groups A and B, there was more disease in proximal than distal segments (p < .05). In groups C and D, which had higher CADS, the degree of coronary artery disease was more uniformly distributed along the vessel.

Table 6 presents the maximal milligrams tension based on concentric or eccentric luminal shape of the coronary segment. The shape of the luminal atherosclerotic plaque had no effect on the maximal milligrams tension generated.

**Effect of heart failure.** The maximal contractile response of the coronary segments to histamine, carbachol, and calcium in normal and failing hearts is given in table 7. Groups A and D represent hearts with normal cardiac function, and groups B and C represent those with severe myocardial dysfunction. There was no difference in the response of the coronary segments to histamine, carbachol, or calcium in these two groups. The numbers of coronary segments given phenylephrine in groups A and D were too few to be compared with those of groups B and C.

**Pharmacologic sensitivity.** Full-contractile dose-response curves to the various vasoactive drugs were constructed from data obtained from segments of the proximal portions of the coronary arteries with a CADS of 1.75 (figures 3A, 3B, 3C, and 4). The rank order of decreasing sensitivity on an M basis (ED_{50}) was phenylephrine > carbachol > histamine > calcium. Dose-response curves derived from segments with a CADS greater than 2.30 were also generated for carbachol, histamine, and calcium. Figure 4 shows the response to histamine. In the segments with a CADS greater than 2.30, the curve is shifted to the left compared with the curve for more normal vessels with a CADS of 1.75, with a 3.3-fold difference in the ED_{50}. In contrast, CADS had no significant effect on the position of the dose-response curves generated for carbachol or calcium (table 8).

**Discussion**

A variety of vasoactive agents contract human coronary vascular smooth muscle. These include histamine, muscarinic agonists, various prostaglandins,
serotonin, \( \alpha \)-adrenergic agonists, and ergonovine.\(^{12}\) All of these agonists are known to operate through combination with specific membrane receptors. Although the physiologic response that results from receptor-agonist combination is dependent on complex cellular mechanisms,\(^{13}\) in vascular smooth muscle calcium flux is a crucial link between receptor activation and muscle contraction.\(^{14}\)

In this and previous studies,\(^{12}\) we present evidence that receptor-mediated contraction of the human epicardial coronary artery is dependent on at least two sources of calcium. One pool is loosely bound and freely exchangeable with calcium in the extracellular space. This "extracellular" pool mediates the contractile response to depolarization, and with the exception of the prostaglandin endoperoxide analog U-44069, accounts for the majority of receptor-activated contraction. Another source of calcium that is used by receptor activation is a tightly bound, slowly exchanging pool that persists after washout in calcium-free solution containing EGTA. This "intracellular" pool is responsible for the majority of U-44069–mediated contraction and mediates less marked but still substantial contraction caused by other agonists. In a normal calcium environment, the administration of a receptor-coupled agonist results in a sustained contraction pattern that is mediated by both calcium pools; in a calcium-free environment only an initial rapid contractile response occurs because calcium is released in the vicinity of the contractile elements. However, this rapid component of contraction is not sustained, since no additional calcium is available to enter the cell from the extracellular space. Thus, it appears that in the human coronary artery, receptor-mediated agonists use at least two distinct pools of calcium for mediating contraction, and the relative extent to which each pool is used is specific for each receptor pathway.

The vasoconstrictive drug generating the greatest maximal tension was the prostaglandin endoperoxide analog U-44069. Other investigators using isolated human coronary arteries have demonstrated contraction in response to several prostaglandins, including PGE\(_2\), PGH\(_2\), PGF\(_{2\alpha}\)\(^{15-18}\) and at high doses, PGI\(_2\).\(^{15,19}\) U-44069 is also unique in that the primary source of calcium used for contraction is from the poorly exchangeable, presumably intracellular, Ca\(^{2+}\) pool. In the aorta from the rabbit, U-44069 has been demonstrated to mediate contraction by activating a calcium influx pathway and by releasing a portion of the intracellular calcium pool.\(^{20}\) This mechanism of action in the aorta of the rabbit is similar to that in the human coronary artery, although it is always possible that U-44069 is also acting directly on the contractile elements.

Histamine is a vasoconstrictive drug that contracts the human epicardial coronary artery through the \( H_1 \) receptor subtype.\(^9\) This endogenous substance generated the second greatest total milligrams tension of all receptor-coupled agonists evaluated. Other agonists such as ergonovine, phenylephrine, and serotonin produced lesser amounts of contractile tension than did U-44069 or histamine. The greater response of many of these agonists compared with potassium depolarization was probably due to the additional contribution of intracellular calcium release. The rank order of maximal contractile tension generated by drugs (U-44069 > histamine > carbachol > serotonin > phenylephrine > ergonovine) was different from the relative potency as determined by the \( ED_{50} \) (phenylephrine > carbachol > histamine). However, all of these drugs

### TABLE 4B

Milligrams tension/square millimeter of media (n = 31 hearts; mean ± SE)

<table>
<thead>
<tr>
<th>CADS</th>
<th>Histamine</th>
<th>Carbachol</th>
<th>Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>874 ± 54</td>
<td>987 ± 68</td>
<td>1221 ± 82</td>
</tr>
<tr>
<td>1.50</td>
<td>499 ± 58</td>
<td>524 ± 73</td>
<td></td>
</tr>
<tr>
<td>2.00</td>
<td>438 ± 67</td>
<td>247 ± 42</td>
<td></td>
</tr>
<tr>
<td>2.50</td>
<td>263 ± 60(^a)</td>
<td>187 ± 54(^a)</td>
<td>293 ± 59(^a)</td>
</tr>
</tbody>
</table>

\(^a\)Statistically different (p < .05) from CADS 1.00.

**TABLE 5**

CADS in proximal, middle, and distal sections of the vessel for groups A to D

<table>
<thead>
<tr>
<th>Group</th>
<th>Proximal</th>
<th>Middle</th>
<th>Distal</th>
<th>( p ) value (( p &lt; .05 ) is significant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>vs middle</td>
<td>vs distal</td>
<td>vs distal</td>
<td></td>
</tr>
<tr>
<td>A Normal</td>
<td>1.85 ± 0.08</td>
<td>1.53 ± 0.20</td>
<td>1.27 ± 0.06</td>
<td>.01</td>
</tr>
<tr>
<td>B Cardiomyopathy</td>
<td>1.81 ± 0.08</td>
<td>1.58 ± 0.03</td>
<td>1.54 ± 0.03</td>
<td>.001</td>
</tr>
<tr>
<td>C Coronary disease</td>
<td>2.40 ± 0.24</td>
<td>2.14 ± 0.17</td>
<td>1.54 ± 0.03</td>
<td>.06</td>
</tr>
<tr>
<td>D Transplant</td>
<td>1.93 ± 0.09</td>
<td>1.76 ± 0.09</td>
<td>1.77 ± 0.21</td>
<td>.20</td>
</tr>
</tbody>
</table>

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produced contraction of coronary artery smooth muscle in concentrations that are achievable in blood or cardiac tissue.

In several different species, acetylcholine mediates relaxation of vascular smooth muscle in vitro. Furchgott and Zawadzki\textsuperscript{21} observed that removal of the endothelium from the isolated blood vessel abolishes the relaxation induced by acetylcholine. Thus the functional integrity of the endothelium may be essential for the maintenance of normal vascular reactivity. In this study, we used ring segments to minimize any potential trauma to the intima when placed in the organ bath. Histologic examination of the segments after the study revealed no disruption of intimal integrity. Carbachol, a muscarinic agonist similar to acetylcholine, produced contraction rather than relaxation in our preparations. Thus, it appears that unlike that of other species, the human coronary artery contracts in the presence of muscarinic stimulation.

The tension developed to each agonist can be altered by the degree of atherosclerosis. When the contractile response to each agonist was analyzed according to

FIGURE 3. Full contractile dose-response curves for phenylephrine (A), carbachol (B), and calcium (C).

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degree of coronary atherosclerosis, alterations in response did occur. For all agonists tested, maximum tension decreased in severely atherosclerotic segments. However, even with advanced disease, the coronary segments were still capable of generating a contractile response to all five agonists evaluated. Intimal infiltration with atherosclerotic disease and minimal media involvement were probably responsible for the continued response in segments with a high CADS. This supports the clinical observation that vessels heavily involved with atherosclerosis are not fixed rigid tubes, but rather remain reactive and are quite capable of contracting in response to vasoactive stimuli.

In moderately diseased coronary segments, the dose-response curve of histamine was shifted to the left, indicating atherosclerosis-induced supersensitivity of the H1 histaminic pathway. A potential explanation for the supersensitivity includes an increase in H1 receptor density in diseased coronary segments, as has been described in the aortas of rabbits fed cholesterol. Alternatively, differences in components of the receptor mechanism between the specific receptor and the effector unit could account for the increased sensitivity of atherosclerotic segments to histamine. However, since the degree of atherosclerosis had no effect on responses to carbachol and calcium, it would appear that the mechanism responsible for histaminic supersensitivity is localized to components of the H1 pathway not shared by the other receptor pathways, and that this mechanism does not involve fundamental changes in the contractile units. Although the clinical significance of atherosclerosis-induced histaminic supersensitivity is unknown, histamine can provoke coronary spasm, and coronary spasm occurs most commonly in atherosclerotic portions of the vessel.

Because of variations in the histologic and angiographic shape and distribution of atherosclerotic plaques, the responses to vasoactive stimuli were analyzed according to morphologic characteristics of the segment. Contractile responses to all vasoactive drugs were similar in segments with concentric and eccentric lesions. Therefore, our findings do not support the concept that morphologic shape of plaque controls the maximal tension that a coronary artery can produce.

Although the majority of hearts used in this study did not have normally functioning myocardium, the purpose of this study was to observe and elucidate the pharmacologic responses of the coronary artery. The presence of heart failure can influence the

### TABLE 6
Maximal response in coronary artery according to shape of luminal atherosclerotic plaque (n = 31 hearts, mean ± SE)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Normal (CADS = 2.0)</th>
<th>Eccentric (CADS = 2.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>940 ± 140</td>
<td>1340 ± 130</td>
</tr>
<tr>
<td>Carbachol</td>
<td>1440 ± 230</td>
<td>1170 ± 60</td>
</tr>
<tr>
<td>KCl</td>
<td>650 ± 130</td>
<td>550 ± 76</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>370 ± 40</td>
<td>630 ± 160</td>
</tr>
<tr>
<td>Ergonovine</td>
<td>250 ± 72</td>
<td>159 ± 30</td>
</tr>
</tbody>
</table>

### TABLE 7
Maximal response to histamine, calcium, and carbachol in failing and nonfailing hearts (mean ± SE)

<table>
<thead>
<tr>
<th>Maximal milligrams tension</th>
<th>Histamine</th>
<th>Carbachol</th>
<th>Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonfailing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>1530 ± 112.1</td>
<td>843 ± 156</td>
<td>1415 ± 797</td>
</tr>
<tr>
<td>Group D</td>
<td>1102 ± 922</td>
<td>708 ± 175</td>
<td>830 ± 562</td>
</tr>
<tr>
<td>Failing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>1180 ± 762</td>
<td>960 ± 120</td>
<td>1403 ± 210</td>
</tr>
<tr>
<td>Group C</td>
<td>1190 ± 552</td>
<td>1080 ± 560</td>
<td>1435 ± 190</td>
</tr>
</tbody>
</table>

Not statistically different from nonfailing hearts.

---

**FIGURE 4.** Dose-response curve to histamine of proximal coronary segments with CADS less than 1.75 and CADS greater than 2.30.
pharmacologic response of myocardial tissue,\textsuperscript{25, 26} but these alterations appear to be confined to adrenergic receptors\textsuperscript{25-27} and appear to be under local rather than systemic control.\textsuperscript{28} Therefore there is no reason to suspect that the presence of heart failure could have altered the responses of coronary artery to histamine, carbachol, or calcium. Moreover, pharmacologic examination of equivalent coronary segments from normally functioning and myopathic failing hearts did not reveal any apparent differences. However, it is always possible that heart failure could have produced subtle changes in quantitative pharmacology that were not detected by comparison with those of the relatively small number of nonfailing hearts examined in this study. This might be especially true for $\alpha$-adrenergic responses, which could theoretically be altered by the long-term exposure to elevated levels of norepinephrine that occur in subjects with heart failure.\textsuperscript{29}

The pharmacologic effects of coronary artery disease described in this study would appear to be generally valid, since the distribution of coronary artery disease along the course of the epicardial vessels was similar to that observed in previous studies conducted in tissue obtained at autopsy.\textsuperscript{30-33} Degenerative disease was most extensive in the proximal portions of the vessels, and the presence of atherosclerosis correlated directly with age, becoming most apparent in the fourth decade of life. There was, therefore, nothing atypical about the type of coronary artery disease observed in this study.

Finally, this study was undertaken because precise quantitative pharmacologic relationships can only be studied in isolated tissue and often provide insights into function in vivo. However, it should be emphasized that pharmacologic responses obtained in isolated tissue did not necessarily apply in vivo. In intact tissue, several important influences are present that cannot be duplicated in vitro, including innervation and multiple humoral factors. Consequently, it will be important to correlate the findings of this investigation with data obtained in vivo.

In summary, the isolated human epicardial coronary artery is a highly reactive vessel capable of contraction in response to a variety of vasoactive drugs. In contrast to the contractile process of cardiac muscle, that of the coronary arteries involves at least two kinetically definable sources of calcium. Use of these calcium pools is specific for each receptor pathway, as is the total amount of tension generated. Atherosclerosis renders the vessel supersensitive to histamine, but otherwise does not substantially alter the physiologic response to most vasoactive drugs until the segment becomes severely diseased. Among classes of vasoactive drugs that may potentially mediate pathologic contractile responses in the human coronary artery, prostaglandins and histamine may be relatively more important than adrenergic agonists.\textsuperscript{34, 35}

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