References and Notes

1. U. Bachrach, *Function of Naturally Occurring Polyamines* (Academic Press, New York, 1973); B. E. Huber and N. A. Brown, *In Vitro* **18**, 599 (1982); D. Russell and S. Snyder, *Biochemistry* **60**, 1420 (1968); J. Jänne, H. Pösö, A. Raina, *Biochim. Biophys. Acta* **473**, 241 (1978).
2. A. E. Pegg and P. P. McCann, *Am. J. Physiol.* **243**, C212 (1982).
3. A. Kallio and P. P. McCann, *Biochem. J.* **200**, 69 (1981); B. W. Metcalf *et al.*, *J. Am. Chem. Soc.* **100**, 2551 (1978).
4. J. R. Fozard *et al.*, *Science* **208**, 505 (1980).
5. O. Heby, *Differentiation* **19**, 1 (1981).
6. D. R. Bethell and A. E. Pegg, *Biochem. Biophys. Res. Commun.* **102**, 272 (1981).
7. A. Altman, R. Friedman, N. Levin, *Plant Physiol.* **69**, 876 (1982); E. Cohen, Y. Heimer, Y. Mizrahi, *ibid.* **70**, 544 (1982); Y. Heimer, Y. Mizrahi, U. Bachrach, *FEBS Lett.* **104**, 146 (1979); R. Kaur-Sawhney, L. Shih, A. W. Galston, *Plant Physiol.* **69**, 411 (1982); A. W. Galston, *BioScience* **33**, 382 (1983).
8. N. Palavan and A. W. Galston, *Physiol. Plant.* **55**, 438 (1982); K. Sen, M. Choudhuri, B. Ghosh, *Phytochemistry* **20**, 631 (1981).
9. H. E. Flores and A. W. Galston, *Science* **217**, 1259 (1982).
10. E. Cohen, S. Arad, Y. Heimer, Y. Mizrahi, *Plant Physiol.* **70**, 540 (1982).
11. M. J. Montague, J. W. Koppenbrink, E. G. Jaworski, *ibid.* **62**, 430 (1978); M. J. Montague, T. A. Armstrong, E. G. Jaworski, *ibid.* **63**, 341 (1979).
12. W. Halperin and D. F. Wetherell, *Am. J. Bot.* **51**, 274 (1964); J. Reinert, *Phytomorphology* **17**, 510 (1967); F. C. Steward, *Am. J. Bot.* **45**, 709 (1958).
13. A. Kallio, P. P. McCann, P. Bey, *Biochemistry* **20**, 3163 (1981).
14. C. Steglich and I. Scheffler, *J. Biol. Chem.* **257**, 4603 (1982); A. Bitonti, P. P. McCann, A. Sjoerdsma, *Biochem. J.* **208**, 435 (1982).
15. R. Kaur-Sawhney, L. Shih, H. E. Flores, A. W. Galston, *Plant Physiol.* **69**, 405 (1982).
16. R. L. Malmberg and J. McIndoo, *Nature (London)* **305**, 623 (1983).
17. We extracted ADC from approximately 100 mg of cells in 1 ml of cold extraction medium containing 10 m*M* Hepes, 1 m*M* dithiothreitol, and 1 m*M* EDTA. The assay was performed at *p*H 7.0, the *p*H at which wild carrot ADC exhibited optimum activity in our reaction system. Ten microliters of DL-[1-^{14}C]arginine (0.5 μCi, 12 mCi/mmole; Research Products International) was used as a substrate in the reaction.
18. S. R. Baker, L. H. Jones, R. J. Yon, *Phytochemistry* **22**, 2167 (1983).
19. G. D. Luk, C. I. Civin, R. M. Weissman, S. B. Baylin, *Science* **216**, 75 (1982); E. O. Niskanen *et al.*, *Cancer Res.* **43**, 1536 (1983).
20. J. Schindler, M. Kelly, P. P. McCann, *Biochem. Biophys. Res. Commun.* **114**, 410 (1983).
21. H. E. Flores and A. W. Galston, *Plant Physiol.* **69**, 701 (1982).

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Coronary Arteries of Cardiac Patients Are Hyperreactive and Contain Stores of Amines: A Mechanism for Coronary Spasm

Abstract. *Coronary arteries from hearts of cardiac patients contain significantly higher concentrations of histamine than do those from noncardiac patients. The coronary vessels of cardiac patients are also hyperresponsive to histamine and serotonin. These differences between groups of patients suggest an explanation for coronary artery spasm in heart disease.*

Coronary artery spasm is now recognized as a clinical entity implicated in heart disease (*1*). A number of reports point to a sudden sustained contraction of a large surface artery feeding the heart muscle in the initiation of some cases of myocardial infarction, angina pectoris, and sudden death (*2*). Coronary spasm has been observed repeatedly during angiographic examination of the hearts of subsets of cardiac patients, but no satisfactory explanation of the vascular derangement that might induce sudden and protracted tone changes and the ensuing myocardial hypoxia and cardiac damage is yet available.

In the study reported here we found that coronary arteries obtained postmortem from patients with a history of coronary artery disease and pathological evidence of myocardial damage respond to biogenic amines with contractions that are significantly larger than those of vessels from patients with no history of cardiac disease. In addition, we found that the coronary vascular tissue from cardiac patients contains stores of these amines, and one of them, namely histamine, is substantially elevated above control values from the arteries of noncardiac patients. We studied the coronary arteries of ten patients whose cause of death was attributed to coronary heart disease. Sudden cardiac death (less than 1 hour) was considered to have occurred in two of these patients. In eight of the ten patients autopsy revealed old or recent infarct damage (scarring or necrosis). In a control group of 18 patients death was attributed to accident (two cases), suicide (one case), carcinomatosis (six cases), hemorrhagic pancreatitis (one case), Hodgkins lymphoma (one case), cerebral or brain stem hemorrhage (three cases), hepatic or renal failure (two cases), atypical pneumonia (one case), and idiopathic aplastic anemia (one case). In only one of the 18 control patients did the postmortem reveal evidence of an old (unreported) infarct.

For biochemical studies portions of the right, left, and circumflex coronary arteries were removed within 10 hours of death (average 6.3 ± 0.5 hours in 20 patients) and immediately placed in chilled (4°C) and previously oxygenated Krebs-Henseleit solution. The tissues were transported to the laboratory in vacuum bottles and carefully placed in fresh, oxygenated and chilled Krebs solution. They were trimmed of all adherent fat and connective tissue, then minced and homogenized in either 5 percent trichloroacetic acid (TCA) (for 5-hydroxytryptamine and histamine) or *n*-butanol (catecholamines) by means of a Kinematica Polytron (full speed for 30 seconds at 0°C). The samples were allowed to stand for at least 10 minutes and then were centrifuged at 10,000*g* for 15 to 20 minutes at 0°C, and the supernatants were stored at −20°C.

5-Hydroxytryptamine (serotonin) was analyzed by the method of Somerville and Hinterberger (*3*), which is based on the development of fluorescence with orthophthaldialdehyde. Total catecholamines were determined by the ethylenediamine condensation method essentially as described by Ogasahara *et al.* (*4*). Histamine was analyzed by a slightly modified version of the method described by Håkanson and Ronnberg (*5*, *6*).

All values were corrected for dilution during the extraction procedures and are expressed as nanograms of amine per gram of wet tissue. The chemical analyses were performed on numbered samples without knowledge of the patient's medical history. At least two coronary vessel segments were assayed from each patient (between 800 and 1000 mg each), and the results were pooled to obtain a single mean value for each patient unless indicated otherwise.

The coronary arteries contained surprisingly high concentrations of serotonin and histamine but a low concentration of catecholamines, the latter probably reflecting a paucity of sympathetic innervation (Table 1). No significant differences between the two groups of patients in the concentrations of serotonin or of noradrenaline plus adrenaline (catecholamines) were detected, but the concentration of histamine was nearly doubled in the arteries of cardiac patients (Table 1). If the data are described on the basis of individual vessel segments, rather than by patients, the level of histamine in 18 vessels from cardiac patients was clearly elevated above that of 27 vessels from noncardiac cases (Table 1). The concentration of serotonin was slightly diminished in the arteries of cardiac patients if values are expressed in terms of individual vessel segments. The values for noradrenaline did not differ between the vessels of the two patient groups.

The differences in the histamine content between cardiac and noncardiac patients was not attributable to postmortem times which averaged 6.4 ± 0.8

Table 1. Concentrations of histamine, serotonin, and catecholamines in the coronary arteries of cardiac and noncardiac patients. Values shown are means ± standard error of the mean; *N* indicates number of patients, or number of vessels, as appropriate. Probabilities were determined by Student's two-tailed *t*-test. N.S., not significant.

Vessel analysis	Histamine			Serotonin			Total catecholamines		
	Concentration (ng/g)	*N*	*P*	Concentration (ng/g)	*N*	*P*	Concentration (ng/g)	*N*	*P*
				Noncardiac patients					
Patients	4544 ± 754	11		5313 ± 635	12		146.7 ± 21.8	6	
Vessels	4457 ± 483	27		5194 ± 524	32		149.7 ± 22.8	14	
				Cardiac patients					
Patients	8619 ± 1274	7	< 0.01	3845 ± 848	8	N.S.	131.8 ± 9.1	4	N.S.
Vessels	8225 ± 1147	18	< 0.01	3455 ± 532	24	< 0.05	129.2 ± 10.2	10	N.S.

hours in the noncardiac and 7.0 ± 0.8 hours in the cardiac patients. Additionally, the histamine concentration of arteries removed as promptly as possible after death (2 to 4 hours) averaged 7048 ± 2226 ng/g and those removed 8 to 10 hours after death averaged 6042 ± 739 ng/g. These values were not significantly different from each other. The reliability of the histamine data was also confirmed in other experiments with cattle hearts. The hearts were transported promptly from the slaughterhouse, to the laboratory (20 minutes) and two left coronary vessel segments were removed from each of two hearts at 30 minutes and 2, 4, and 8 hours after death of the animals and assayed for histamine. No significant differences in the concentrations of histamine were apparent. The values of 16821 ng/g at 30 minutes and 19200 ng/g at 2 hours did not differ significantly from the value of 17690 ng/g recorded at 8 hours.

The ages of the patients in the cardiac and noncardiac groups assayed for histamine did not differ significantly, being 66.1 ± 4.2 years and 57.7 ± 4.4 years. The concentrations of coronary histamine in males and females, regardless of the cause of death, did not differ, being 5551 ± 644 for ten males and 6529 ± 1133 ng/g in eight females.

The vessel segments, used for the analyses of amines, were assessed as atherosclerotic or nonatherosclerotic on the basis of the presence, in the former, of lesions, calcification, and gruel. This assessment was done by visual and tactile inspection at the time the samples were weighed and coded, essentially as outlined by Guzman *et al.* (*7*). Although this procedure is not entirely objective, two investigators agreed on the assessments, which in almost all cases were easily made. The concentrations of serotonin and catecholamines did not differ significantly between the atherosclerotic and nonatherosclerotic groups, but the concentration of histamine was significantly elevated ($P < 0.01$; Student's *t*-test) in the vessel segments assessed as atherosclerotic; the mean values were 7232 ± 742 ng/g (15 tissues) and 4889 ± 439 ng/g (30 tissues), respectively.

Other experiments were done specifically to determine if coronary artery segments from cardiac and noncardiac patients respond differently to histamine, serotonin, and noradrenaline. For this study, ring preparations (6 mm wide) of circumflex and right or left anterior descending arteries, or proximal regions of their major branches, were prepared by trimming off adherent fat and connective tissue and placing two 28- to 30-gauge wire supports through the lumen of the rings, as described (*8*). The lower wire attached the arterial segment to an aeration rod in the muscle chamber and the upper supporting wire was connected by thread to a force-displacement transducer, under 2 g of tension, for isometric recording with a Grass polygraph. The muscle baths were of 15-ml volume and contained Krebs-Henseleit solution at 37°C which was continuously oxygenated (95 percent O_2 and 5 percent CO_2), and the tissues were permitted to equilibrate for a minimum of 90 minutes before agonist testing.

Spontaneous phasic activity and varying degrees of spontaneous tone were observed in a number of preparations from cardiac and noncardiac patients. Usually two or three ring segments from each patient were exposed to each of the test agonists, over a broad concentra-

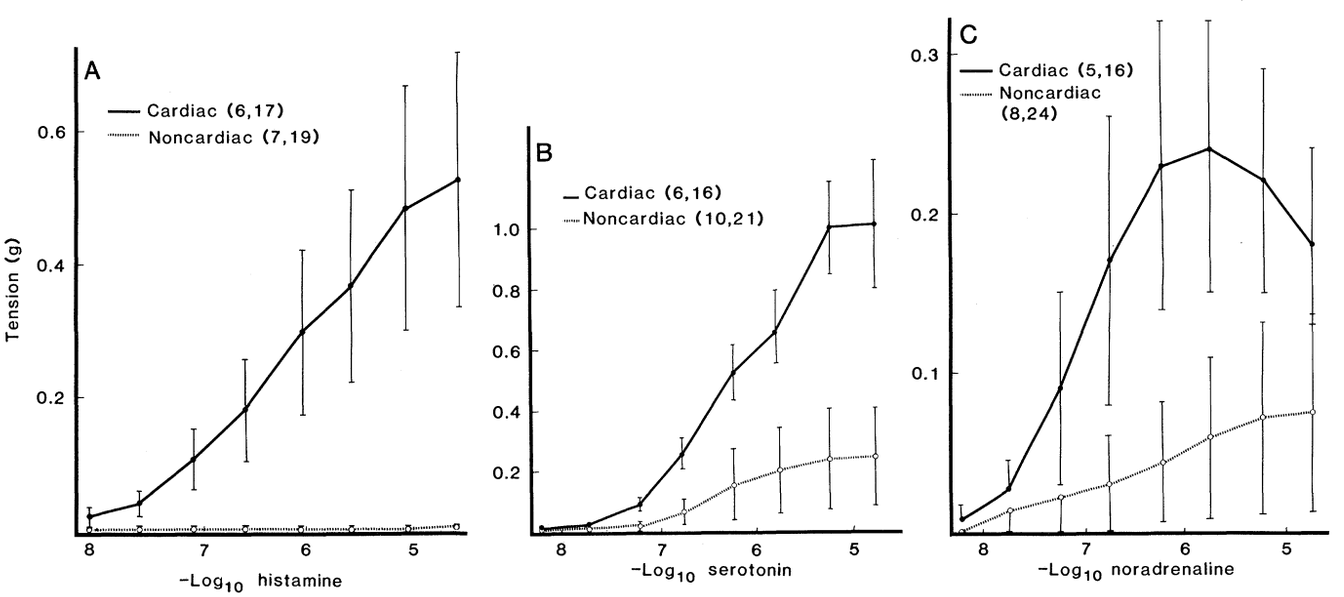

Fig. 1. The response of coronary arteries from cardiac and noncardiac patients to cumulative additions of (A) histamine, (B) serotonin, and (C) noradrenaline. Values in parentheses show, first, numbers of patients and second, numbers of vessel segments, which comprise the mean curve. In each case, the mean concentration-response curve for each patient was obtained by averaging the responses of at least two segments to a given agonist. These, in turn, were averaged to obtain the mean values for the cardiac and noncardiac groups. Values are shown with their standard error bars.

tion-response range, and the results averaged to obtain a single concentration-response curve for each agonist from each patient. These values were then averaged to obtain the mean concentration-response curves for the cardiac and noncardiac groups.

Histamine was administered in cumulatively increasing concentrations from 1×10^{-8} to $3 \times 10^{-5} M$ to 19 ring segments from seven noncardiac and 17 ring segments from six cardiac patients. As shown in Fig. 1 the vessels from cardiac patients responded with markedly greater contractions to histamine than did those from noncardiac patients. The maximal response of 0.53 ± 0.19 g was over 1000-fold greater than that of the noncardiac group. Contractions in response to serotonin were also substantially increased in vessels from cardiac patients (Fig 1): the mean maximal response was 458 percent greater than in the noncardiac group. Responses to noradrenaline also appeared to be increased in vessels from cardiac patients (Fig. 1). The functional form of these curves is not known so that one cannot define the correct statistical test for comparing the two curves. Statistically significant differences can, of course, be shown for approximations to the curves. The maximal responses of the two groups of preparations to noradrenaline were 0.29 ± 0.12 g and 0.07 ± 0.04 g ($P < 0.05$).

The differences between cardiac and noncardiac patients in their coronary vascular responses to agonists could not be attributed to differences in the times between death and postmortem because they were comparable in the two categories (for example, 5.4 ± 0.5 hours and 4.9 ± 1.1 hours in the tissues used for histamine). The ages of the patients in the cardiac and noncardiac groups did differ significantly, and this reflected the high frequency of cardiac disease in the population of older patients sampled during the period of this study. Ages of the cardiac and noncardiac groups averaged 69.5 ± 4.4 years and 45.1 ± 6.6 years, respectively, for the histamine-treated and 69.5 ± 4.4 years and 44.1 ± 5.2 years for the serotonin-treated groups.

Three of the cardiac patients had been treated with digoxin and six of them with a combination of drugs for acute myocardial infarct (for example, morphine, lidocaine, diuretics). Three of the patients were under long-term hypertensive drug therapy (for example, diuretics, α-methyldopa) prior to admission. Although the involvement of the drug therapy in the outcome of the experiments described here cannot be entirely ruled out, it is unlikely. One would expect that, since most such drugs interact primarily with adrenergic mechanisms, the tissues would have shown altered catecholamine concentrations and their responsivity changes to noradrenaline would have been most evident, and such was not the case.

The contractile status of the large surface arteries of the heart has assumed rapid clinical importance in the definition and therapy of several major forms of heart disease (*9, 10*). Spasm of a conduit coronary artery with its resultant interference with myocardial blood flow, upstream from the potent compensatory dilator forces of anoxic metabolism in myocardial tissue, may have disastrous consequences for cardiac function and hence the individual. The present results revealed remarkable differences in the responses of the epicardial coronary arteries from cardiac and noncardiac patients to vasoactive substances. The enhanced reactivity of the epicardial arteries of cardiac and older patients observed here appears to provide a background against which a number of vasoactive agents might induce spasm.

Angiographically demonstrable coronary artery spasm was recently induced in miniature swine with experimentally induced atherosclerotic lesions, but not in control pigs, by the intravenous or intracoronary administration of histamine (*11*). Such data support the present findings with human vessels. Also, there is a suggestion that coronary vessel tone, in response to the cold-pressor test, increases more readily in patients with ischemic heart disease than in normals (*12*). Other workers have noted that the cold-pressor test, which evokes systemic vasoconstriction, elicited spasm during arteriography, at the site of an atheromatous plaque, in several patients with diverse chest pain syndromes (*13*), pointing to an increased reactivity of vascular tissue in these patients.

Our finding that coronary tissue from cardiac patients contains stores of histamine that are substantially increased above values in noncardiac patients, and that the vascular tissue also contains serotonin, suggests that a sudden release of vasoactive material, due to injury or an "allergic" response as occurs in an antigen-antibody type reaction (*14*), could induce a powerful contraction or spasm of a coronary vessel segment and precipitate a cardiac crisis such as angina or rhythm disruption.

In this regard, the location of histamine in the vascular wall of several species (for example, dog limb, renal, and splenic arteries), except the cow, is unclear and appears to involve primarily nonmast cell storage (*15, 16*). However, mast cells have been observed by histological techniques in human coronary artery specimens and they appear to increase "proportionately with the degree of atheroma" (*17*). Pollak (*18*) noted that "adventitial cells [mast] were more numerous around atheromatous vessels than around normal ones." Attempts were made in the present study to release histamine from storage depots in the human coronary vessels with compound 48/80 (Sigma) at concentrations of 30 to 100 μg/ml, but this was unsuccessful as assessed by the unaltered contractile status of the vessels. However, epicardial coronary vessels from cattle responded with slow progressive contractions to compound 48/80, suggesting the release of stored mediators (*19*). Although postmortem measurements of amine concentrations in human brain have been described (*20*), to our knowledge this is the first such report on the coronary vessels of the human heart.

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References and Notes

1. C. R. Conti, *N. Engl. J. Med.* **309**, 238 (1983).
2. S. Kalsner, in *The Coronary Artery*, S. Kalsner, Ed. (Croom Helm, London, 1982), p. 551.
3. B. Somerville and H. Hinterberger, *Clin. Chim. Acta* **65**, 399 (1975).
4. A. Ogasahara *et al.*, *J. Chromatogr.* **180**, 119 (1979).
5. R. Håkanson and A-L. Ronnberg, *Analyt. Biochem.* **60**, 560 (1974).
6. A portion of the TCA extract was washed with 15 ml of water-saturated diethyl ether and the aqueous phase made alkaline with 0.5 ml of 0.5*N* NaOH and shaken with 0.5 g of Na_2SO_4 and 15 ml of butanol-chloroform mixture (3:2 by volume). The organic phase was then washed with 4 ml of 0.1*N* NaOH saturated with Na_2SO_4. Histamine was recovered from the butanol-chloroform mixture by shaking with 3.0 ml of 0.1*N* HCl and 5.0 ml of heptane. A portion of this extract was then neutralized with 1*N* NaOH, mixed with 0.2 ml of 0.1 percent (weight to volume) orthophthaldialdehyde in methanol and 1.0 ml of 0.1*N* NaOH, and incubated for exactly 4 minutes at room temperature. The reaction was halted by the addition of 0.2 ml of 0.5*N* H_2SO_4, the mixture was allowed to stand for 10 minutes, and the fluorescence was read at 355 nm (excitation) and 400 nm (emission).
7. M. A. Guzman *et al.*, *Lab. Invest.* **18**, 495 (1968).
8. R. W. Jelliffe, *J. Pharmacol. Exp. Ther.* **135**, 349 (1962).
9. A. Maseri *et al.*, *N. Engl. J. Med.* **299**, 1271 (1978).
10. H. R. Hellstrom, *Br. Heart J.* **41**, 426 (1979).
11. H. Shimokawa *et al.*, *Science* **221**, 560 (1983).
12. G. H. Mudge *et al.*, *N. Engl. J. Med.* **295**, 1333 (1976).
13. A. E. Raizner *et al.*, *Circulation* **62**, 925 (1980).
14. N. Chand and P. Eyre, *Agents and Actions* **8**, 171 (1978).
15. T. M. El-Ackad and M. J. Brody, *Blood Vessels* **12**, 181 (1975).
16. M. J. Ryan and M. J. Brody, *J. Pharmacol. Exp. Ther.* **181**, 83 (1972).
17. A. Pomerance, *J. Pathol. Bacteriol.* **76**, 55 (1958).
18. O. J. Pollak, *Circulation* **16**, 1084 (1957).
19. S. Kalsner, unpublished data.
20. I. J. Farley *et al.*, *Science* **200**, 456 (1978).
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