Psychophysical Responses to a Speech Stressor: Correlation of Plasma Beta-Endorphin Levels at Rest and After Psychological Stress With Thermally Measured Pain Threshold in Patients With Coronary Artery Disease

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Objectives. We tested the hypothesis that psychological stress alters plasma levels of opioid peptides and that these plasma levels are related to pain perception in patients with coronary artery disease.

Background. Public speaking psychological stress has previously been shown to be associated with silent ischemia.

Methods. After instrumentation and a 30-min rest period, venous blood samples for beta-endorphin were obtained before and immediately after psychological stress in 20 patients with coronary artery disease. Pain threshold was then assessed using a thermal probe technique at baseline and immediately after stress. Patients gave three brief speeches lasting a total of 15 min about real-life hassle situations.

Results. Psychological stress significantly increases plasma beta-endorphin levels (4.3 ± 0.9 pmol/liter [mean $\pm$ SE] at rest to 8.3 ± 2 pmol/liter after stress, $p < 0.05$). There was a significant positive correlation between pain threshold and beta-endorphin levels after stress ($r = 0.577$, $p = 0.008$). This significant positive correlation was still present while rest blood pressure and change in blood pressure during stress were controlled for by analysis of covariance techniques.

Conclusions. In patients with coronary artery disease and exercise-induced ischemia, public speaking produces psychological stress manifested by increased cardiovascular reactivity and causes an increase in plasma beta-endorphin levels that is significantly correlated with pain thresholds. These findings may explain the predominance of silent ischemia during psychological stress in patients with coronary artery disease.

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We previously reported (1) a comparison of beta-endorphin levels in patients with coronary artery disease and exercise-induced ischemia both at rest and after stress and found differences in the stress response between patients with silent and symptomatic ischemia and differences between psychological and exercise stress. We further hypothesized that psychological stress-induced ischemia may be more frequently silent because it is associated with a greater increase in plasma beta-endorphin per unit of change in double product (1). However, our previous work was performed without simulta-

neously measured pain thresholds. Because of the controversy about the relation between peripheral plasma beta-endorphin levels and pain perception, we undertook the present study to further examine the hypothesis previously reported and to examine the relation between circulating beta-endorphin levels both at rest and after stress and objective measurements of pain perception utilizing a thermal psychophysical procedure.

Methods

Patients participating in the study were recruited from the inpatient and outpatient cardiology services of University of North Carolina Hospitals as well as local outpatient cardiac rehabilitation programs. The protocol was approved by the Committee on the Protection of the Rights of Human Subjects at the University of North Carolina, and written informed consent was obtained for each subject. Twenty patients (mean [$\pm$SE] age 60 ± 2 years, range 47 to 78) with coronary artery disease and positive exercise test results were studied. Criteria for coronary artery disease were at least one of the following: 1) arteriographically proved diameter narrowing <70% in at

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                                                        at least
------30 min---->    --5 min->   ----10 min--->   --------15 min------->   ------10 min---->
                                                  (2' prep + 3' speech) x 3

    Rest period        Thermal      Resting        Continuous speech task        Rest
                       pain test    radionuclide        with RNV
                                    ventriculo-
                                    gram (RNV)      during          during
                                                     min             min
                                                     8-10            13-15

 Heparin lock          Blood drawn                  Blood drawn
 inserted;            for ß-endorphin             for ß-endorphin
 ambulatory ECG       measurement                  measurement                 Blood drawn
 & BP recorders                                                               for ß-endorphin
 placed                                                                        measurement;
                                                                               heart rate,
                                                              Blood drawn      blood pressure,
                                                            for ß-endorphin    & ECG return
                                                             measurement        to baseline
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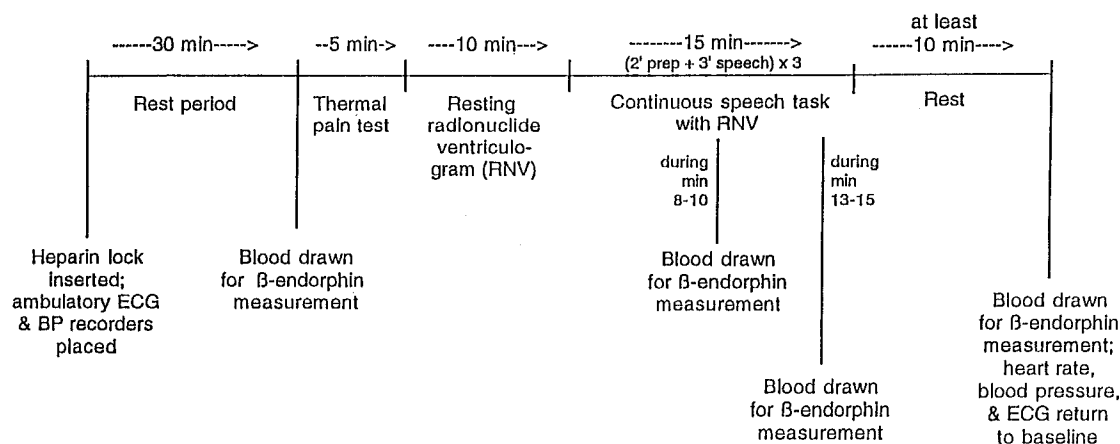
Figure 1. Psychological stress session protocol. BP = blood pressure; ECG = electrocardiogram; prep = preparation.

least one major coronary artery; 2) previous myocardial infarction documented by at least two of the following criteria— typical pain, evolutionary electrocardiographic (ECG) changes or significant elevations of creatine kinase-MB fraction in serum (more than two times normal); 3) typical angina and positive exercise test results (≥ 1 mm horizontal or downsloping ST segment depression at 0.08 s after the J point) with a >90% posttest likelihood of disease based on Diamond and Forrester (2) criteria; 4) positive radionuclide study (failure to increase left ventricular ejection fraction $\geq 5\%$ and development of segmental wall motion abnormalities on blood pool scan or reversible perfusion defects on thallium scan).

Pain assessment/pain threshold and tolerance. Thermal pain threshold and pain tolerance were determined by utilizing a thermal probe technique (3). Thermal stimuli ranging from 40 to 50°C were delivered to the volar forearm with a contact thermode. Each temperature was delivered for 5 s in an ascending series and 0.5°C increments. The delivery of stimuli was under computer control. Patients reported when they first considered the stimulus to be painful (threshold) and when the stimulus reached their pain tolerance level (tolerance). Three ascending series of temperatures were presented to different areas of the volar forearm. Average threshold and tolerance levels were then determined. This thermal probe technique has been shown to be an appropriate noxious stimulus for testing purposes (4). Thermal stimuli provide a quantified stimulus that can be varied between threshold and tolerance, stimulate a restricted group of nociceptors in the pain sensitivity range and provide a quality of stimulus that is constant across pain sensitivity range in humans (4). To assess reproducibility in baseline control measurements, baseline pain threshold and tolerance were determined on two separate days at the same time of day.

Psychological stress protocol. A 12-lead ECG was recorded immediately before, each minute during and for at least 8 min after stress. Leads II, V_4 and V_5 were continuously

monitored. An ambulatory ECG was placed during the rest period and timer synchronized so that accurate analysis could be subsequently performed. Leads V_5 and II were monitored. The event recorder was pressed at specific times in the protocol so that ECG data could be carefully compared with other data obtained. Significant ischemia on the ECG was defined as 1 mm horizontal or downsloping ST segment depression at 0.08 s after the J point. Figure 1 shows the time line for the experimental session. The psychological stress test consisted of continuous preparation for and presentation of three brief tape-recorded speeches involving a real-life hassle situation (the unwanted house guest; a relative being mistreated in a nursing home; and dealing with a store that will not honor an advertised special). A brief description of each of the three topics was read to him or her. The patient was then allowed 2 min to prepare and 3 min to deliver the speech into the microphone of a tape recorder. Two minutes of preparation time for the second speech then commenced, and the same sequence was repeated with no pauses to permit recovery, so that the 5-min preparation/speaking period repeated three times constituted a 15-min stress-inducing task. An audience of three people (two familiar and one stranger, including members of both genders) was present and maintained eye contact with the speaker throughout. To reduce habituation, just before the second speech, the assistant informed the speaker that he or she would receive a small cash bonus ($5) if the second speech was rated equal to or better than the first by the audience. The same instruction with a bonus of $10 was given before the third speech. Blood pressure and heart rate were monitored at 1-min intervals by an automated manometric blood pressure cuff (Accutor). At completion of the task the patient was asked whether chest pain occurred during the preparation period or the speech, and this was recorded as a dichotomous outcome. Pain testing was repeated immediately after stress (within 5 min).

Beta-endorphin measurement. After instrumentation and a 30-min rest period, venous blood samples for beta-endorphin measurement were obtained immediately before and immediately after stress testing. Specimens were collected into tubes containing ethylenediaminetetraacetic acid (EDTA). After

Table 1. Clinical and Angiographic Data

History of Angina	History of Myocardial Infarction	Diseased Vessels on Catheterization	History of Hypertension	Medications
Y = 16 (80%)	Y = 5 (25%)	1 vessel = 8	Y = 12 (60%)	BB = 9 (45%)
N = 4 (20%)	N = 15 (75%)	2 vessel = 2	N = 8 (40%)	CA = 10 (50%)
		3 vessel = 2		LA = 12 (60%)

Data presented are number (%) of patients. BB = beta-adrenergic blocking agent; CA = calcium channel blocking agent; LA = long-acting nitrate; N = no; Y = yes.

centrifugation, the plasma was aspirated and immediately frozen at −70°C until assayed. All samples were assayed in duplicate for beta-endorphin concentration using a radioimmunoassay kit (INCSTAR Corporation). The recovery yield from plasma determined using internal standards ranged between 80% and 106%. The antibody cross-reacts 100% with human beta-endorphin, shows <5% cross-reactivity with beta-lipotropin and has no cross-reactivity with related peptides and hormones, such as alpha-endorphin, gamma-endorphin, leucine enkephalin, methionine enkephalin or adrenocorticotrophic hormone (ACTH). The sensitivity of the assay, defined as the apparent concentration at three standard deviations from counts at maximal binding, is <3 pmol/liter. The intraassay and interassay coefficients of variation were 5.76% and 17.1%, respectively.

Statistical methods. Descriptive statistics are expressed as mean value ± SE throughout the text and in the tables. Significant changes in hemodynamic and beta-endorphin values from before to after stress or rest to peak stress were assessed with paired t tests and Wilcoxon signed rank tests. Pearson correlations were used to assess the relation between pain threshold and beta-endorphin levels and with the square root of beta-endorphin levels to address the relative influence of large values. Evaluation of the relation to the square root of beta-endorphin levels with adjustment for rest blood pressure and change in blood pressure during stress was accomplished with adjusted slopes from multiple linear regression analyses.

Results

Clinical and hemodynamic data. Table 1 shows clinical and angiographic data: 16 patients (80%) had a history of stable angina pectoris, and 9 (45%) had experienced an anginal episode within 3 weeks before the study. Five patients (25%) had experienced myocardial infarction ≥3 weeks before the study. Twelve patients (60%) had undergone cardiac catheter-

ization, which revealed three-vessel disease in two (16%), two-vessel disease in two (16%) and one-vessel disease in eight (67%). Twelve patients (60%) were diagnosed with or being treated for hypertension.

No patient had valvular heart disease or major systemic illness, uncontrolled hypertension, orthopedic or peripheral vascular disease precluding bicycle exercise or was pregnant. Patients were excluded for conditions known or suspected to influence pain perception (diabetes mellitus, alcohol abuse, previous coronary surgery) or to decrease the specificity of exercise-induced ST segment depression (digitalis preparations, left bundle branch block or left ventricular hypertrophy on baseline ECG). Eighteen of 20 patients were studied while taking cardiac medications (beta-adrenergic blocking agents, calcium antagonists, long-acting nitrates). Previous work (1) has shown no effect of these medications on beta-endorphin levels or responses to stress.

Table 2 shows hemodynamic data. During the psychological stress protocol, systolic blood pressure increased from 138 ± 4.01 mm Hg at rest to 180 ± 4.01 mm Hg at peak stress, and heart rate increased from 65 ± 2.93 beats/min at rest to 78 ± 3.59 beats/min at peak stress. These values corresponded to a mean rest rate-pressure product of 9,047 ± 508 and a mean peak product of 14,240 ± 844. No patient had chest pain during psychological stress. Ten of 20 patients had evidence of stress-induced ischemia on the basis of ECG or radionuclide criteria.

Beta-endorphin and pain threshold results. Table 3 summarizes the beta-endorphin and pain threshold results. Mean baseline plasma beta-endorphin level was 4.30 ± 0.90 pmol/liter, which increased to 8.33 ± 2.11 pmol/liter immediately after stress. This mean absolute change (4.03 ± 1.89 pmol/liter) was significant at p = 0.012. This result represents a clinically significant difference of the magnitude that one would be able to detect with a clinical dose of morphine (5). There was no significant difference in baseline pain thresh-

Table 2. Hemodynamic Data Obtained During Psychological Stress Testing

	Baseline	Peak Stress	Peak Stress − Baseline
Systolic blood pressure (mm Hg)	138 ± 4.01	180 ± 4.01	42 ± 3.97
Heart rate (beats/min)	65 ± 2.93	78 ± 3.59	13 ± 2.20
Rate-pressure product	9,047 ± 508	14,240 ± 844	5,193 ± 611

Data presented are mean value ± SE.

Table 3. Pain Threshold and Beta-Endorphin Results

	Baseline	After Stress	After Stress − Baseline	p Value
Pain threshold (°C)	45.0 ± 0.49	45.4 ± 0.46	0.4 ± 0.3	NS
Beta-endorphin (pmol/liter)	4.30 ± 0.90	8.33 ± 2.11	4.03 ± 1.89	0.012 (WS)

Data presented are mean value ± SE. WS = Wilcoxon signed rank test.

old determined on two separate days ($p = 0.299$). Baseline pain threshold was 45.0°C, which increased slightly but not significantly after stress ($p = 0.18$). There was no significant correlation between baseline pain threshold and beta-endorphin levels ($r = 0.163$, $p = 0.492$). There was a significant positive correlation between pain threshold and plasma beta-endorphin levels after psychological stress ($r = 0.577$, $p = 0.008$). Because threshold was normally distributed and endorphin values were skewed, we also correlated threshold with square roots of endorphin levels ($r = 0.460$, $p = 0.041$ for square roots, slope 0.668 ± 0.304 SE). This significant positive relation was still present in multiple linear regression analyses controlling for rest blood pressure (slope 0.689 ± 0.308 SE, $p = 0.04$) and change in blood pressure during stress (slope 0.667 ± 0.306 SE, $p = 0.04$).

Discussion

Significance of plasma opioid levels. There is considerable controversy in published reports regarding the significance of peripheral plasma levels of opioid peptides in mediating changes in pain perception and in the significance of cutaneous pain thresholds as adequate indicators of the status of visceral nociception, particularly as it relates to patients with cardiac ischemia.

Several investigators (6–8) have reported that plasma opioid peptide levels positively correlate with pain perception indexes measured from cutaneous tissues. Others have not found such a relation (9,10). These reported differences may be due to many factors, including differences in patient populations, such as possible gender differences to psychological stress (11), differences in assay techniques, differences in experimental protocol that might affect an adequate baseline status and differences in level of stress produced, which affect the degree of change in levels of opioid peptides and pain perception, and differences in the type of stressor applied (exercise vs. mental stress). In addition, not all types of pain-testing procedures are equivalent. For example, electrical pain testing is a nonspecific stimulus that activates many types of cutaneous receptors, whereas thermal pain testing more selectively stimulates neuronociceptive pathways (4).

We previously showed differences between the plasma beta-endorphin response to exercise versus psychological stress (1) but did not measure pain thresholds. The present study confirms our previous work in that it shows significant increases in plasma beta-endorphin levels in response to a psychological stressor and in addition demonstrates that thermal pain thresholds after stress are significantly correlated with beta-endorphin levels ($r = 0.58$, $p = 0.008$).

Some doubt the significance of peripheral levels of opioid peptides in adequately reflecting pain perception. The arguments given for this opinion are 1) the inconsistency in the reports previously cited; and 2) that beta-endorphin in the plasma does not cross the blood–brain barrier and therefore cannot be important in modifying nociception, because many believe that nociception is controlled entirely by central nervous system mechanisms.

Circulating endorphin levels appear to influence pain perception and nociceptive reflexes (12), although this link has not been clearly established or its mechanism elucidated. It is thought that opiates may act peripherally by suppressing the release of algesic substances from primary afferents (13) or by stimulating specific opiate receptors on cardiopulmonary vagal afferents (14). A link between the cardiovascular baroreceptor reflex arc and pain regulatory systems in both animals and humans has been found (15,16), suggesting that in humans the clinical effect of baroreceptor stimulation may modify pain regulatory systems. However, the positive correlation between beta-endorphin levels and pain threshold after stress in the present study was still present while blood pressure change during stress was controlled.

Baroreceptors and pain perception. A preliminary study by our group (17) suggests that stimulation of the baroreceptors may modify perception of myocardial ischemia pain in patients with coronary artery disease. Twenty-eight patients with coronary artery disease who had demonstrated angina and positive exercise ECG findings underwent supine exercise radionuclide angiography. An index of ischemic pain perception was calculated by determining time from the start of exercise to the onset of 1-mm ST segment depression and subtracting that value from the time to the onset of angina. This index was positively correlated with systolic blood pressure at rest ($p = 0.03$), and both the index and rest blood pressure correlated negatively with duration of angina ($p < 0.01$ and $p < 0.001$, respectively).

Recently we showed that beta-endorphin plasma infusion modifies the nociceptive response to adenosine infusion (Sylven L, Sheps DS, unpublished observations). Adenosine is thought to be the major chemical mediator of ischemic cardiac pain, and therefore demonstration of an antinociceptive effect of intravenous beta-endorphin infusion in humans provides further proof that beta-endorphin exerts a physiologic effect in the peripheral venous blood. Another opioid peptide that is secreted peripherally in response to stress by the adrenal

medulla is met-enkephalin. We did not measure met-enkephalin in the present study, but it is also known to be an important opioid peptide involved in modulation of antinociception. It has also been recently demonstrated to be present in cardiac myocytes and may play an important role in mediating localized physiological responses (18).

Relevance of psychological stress. It must be emphasized that results obtained in the present study apply solely to a psychological stressor (public speaking) and cannot be assumed to be applicable to the situation that occurs with exercise stress. The hemodynamic and neuroendocrine responses to exercise stress are different from those associated with psychological stress (lower epinephrine responses to exercise stress and more gradual blood pressure change), and therefore the relation described in the present study dealing with psychological stress may not be obtained with exercise.

The importance and relevance of laboratory-evoked responses to psychological stress have been studied by Matthews et al. (19) who found that some cardiovascular reactors to a speech stressor in the laboratory had greater ambulatory systolic and diastolic blood pressure levels during periods of stress (during ambulatory monitoring) than during periods of nonstress. These differences were more than four times those of the nonlaboratory reactors. Thus, the daily life implications of a laboratory speech stressor are significant in at least some subjects.

It has been shown that silent ischemia during daily life in patients with coronary artery disease is associated with an adverse prognosis. It is logical to postulate that patients who have greater blood pressure changes with psychological stress may also have more ambulatory silent ischemia, although to our knowledge this has not been tested. Thus, psychological stress-induced ischemia may also be associated with an adverse prognosis in patients with coronary artery disease. This hypothesis can be proved with a prospective study that follows up patients with ischemic heart disease after a psychological stressor.

Summary. We showed that a public speaking psychological stressor produces increases in plasma beta-endorphin levels that are correlated with peripheral pain threshold in patients with coronary artery disease. This relation provides further support for an opioid mechanism for psychological stress-induced silent ischemia. The possible adverse clinical effect of such stressors occurring during daily life remains to be determined.

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