

Heterogeneity in Neuroendocrine and Immune Responses to Brief Psychological Stressors as a Function of Autonomic Cardiac Activation

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Human responses to brief psychological stressors are characterized by changes and large individual differences in autonomic, neuroendocrine, and immune function. The authors examined the effects of brief psychological stressors on cardiovascular, neuroendocrine, and cellular immune response in 22 older women to investigate the common effects of stress across systems. They also used interindividual variation in heart rate reactivity, cardiac sympathetic reactivity (as indexed by preejection period reactivity in their reactivity paradigm), and cardiac vagal reactivity (as indexed by respiratory sinus arrhythmia reactivity) to explore the heterogeneity in human responses to brief psychological stressors. The results revealed that brief psychological stressors heightened cardiac activation, elevated plasma catecholamine concentrations, and affected the cellular immune response. It was also found that individuals characterized by high, relative to low, cardiac sympathetic reactivity showed higher stress-related changes in adrenocorticotrophic hormone and cortisol plasma levels but comparable changes in epinephrine and norepinephrine concentrations. These data suggest that the effects of psychological stressors on cardiovascular and cellular immune response are governed by coordinated regulatory mechanism(s) and that going beyond the simple notion of heart rate reactivity to examine neural substrates may shed light on the interrelationships among and the regulatory mechanisms for the autonomic, endocrine, and immune responses to stressors.

Key words: Psychological stress, heart rate reactivity, preejection period, sympathetic reactivity, neuroendocrine response, cellular immune response.

INTRODUCTION

Brief psychological stressors are virtually universal; can be emulated in the laboratory; and have clear psychological, autonomic, and neuroendocrine effects. The immunological consequences of brief psychological stressors have only recently begun to be studied, however (for reviews, see Refs. 1 and 2). Although the results from these studies reveal some consistency in effects, different subsets of immune measures have tended to be used, which makes comparisons across studies difficult. Furthermore, some of the autonomic and neuroendocrine links to

the immune system have been outlined (3–6), but empirical data on the interrelationships among autonomic, neuroendocrine, and immune responses to brief experimental stressors in humans are limited (7–9). The first aim of our study, therefore, was to investigate more comprehensively the general effects of brief psychological stressors across these systems.

A second major aim was to investigate the heterogeneity in people's response to psychological stressors. Research by Manuck et al. (8) and Bachen et al. (10) provided evidence that individuals who exhibit relatively high cardiovascular and/or catecholaminergic reactivity also exhibit larger immune responses to stress. Congruent with the involvement of the sympathetic adrenomedullary system in immunological regulation (11), Manuck et al. (8) reported that psychological stressors elevated plasma epinephrine (EPI) and norepinephrine (NE) levels, were associated with a decrease blastogenic response of peripheral blood leukocytes (PBLs) to mitogens, and an increase in the percentage of natural killer (NK) cells and NK cytotoxicity. These authors therefore posited that acute psychological stress activates the sympathetic adrenomedullary axis.

Consistent with the suggestion by Manuck et al. (8), the extant research indicates that brief psychological stressors elevate sympathetic activity and plasma catecholamine levels (9, 12–14) and that

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stress-related increases in heart rate and cardiac systolic time intervals are abolished by β -adrenergic blockade (15). The effect of brief psychological stressors on the hypothalamic pituitary adrenocortical axis is less clear, however. The pituitary adrenocortical system is governed by hypothalamic mechanisms and chemical messengers (e.g., corticotropin-releasing hormone) that can affect autonomic activity (16), and glucocorticoids have been shown to have immunoregulatory effects (17). Although Manuck et al. (8) and Bachen et al. (10) found the cortisol level was unaffected by brief psychological stressors, Lovallo et al. (18) reported that high-, but not low-, heart rate (HR) reactors showed significant elevations in plasma cortisol concentrations with a 15-minute aversive psychological stressor. Similarly, we found that high-HR reactors were characterized by higher stress-related plasma cortisol concentrations with a 12-minute mental arithmetic and noise stressor (9). Lovallo et al. (18) and we (9) also observed that the psychological stressors produced comparable increases in NE levels for high- and low-HR reactors. These results are congruent with Frankenhaeuser et al.'s hypothesis that NE responses are related to the amount of effort required in a task, whereas cortisol responses are related to the amount of threat or distress produced by a task (19). To investigate individual differences in the neuroendocrine changes further that occur during brief psychological stressors, however, we measured catecholaminergic (EPI and NE), adrenocorticotrophic hormone (ACTH), and cortisol responses to a brief (12-minute) aversive psychological stressor.

In light of the data that demonstrate that catecholamine infusion decreases blastogenic responses of PBLs to mitogens and increases NK cell percentage and NK cytotoxicity, we were less interested in studying individual differences in catecholaminergic activation than in determining whether interindividual variability in cardiac reactivity would predict differences in neuroendocrine and immune response. There were several reasons for our interest in interindividual variations in cardiac reactivity. First, stable and generalizable individual differences in cardiac reactivity are identifiable when the reactivity assessment is based on multiple measures of cardiac activity during baseline and stressor periods (20–25). Second, HR reactivity has been implicated in prior research as a possible discriminator of individual differences in neuroendocrine and immune response to stress (7, 9). For instance, Knapp et al. (7) examined autonomic and immune responses while subjects recalled and imagined maximally disturbing and maximally pleasurable emotional experi-

ences. Correlational analyses between changes in autonomic and immunological variables during the negative emotional task revealed that HR correlated negatively and significantly with the blastogenic response of PBLs to phytohemagglutinin (PHA), and it correlated positively and significantly with NK cell activity and total lymphocyte numbers. In a study of undergraduate men, we recently identified 11 high and 11 low reactors based on the extremes of the HR reactivity distribution to a speech stressor in a prescreening session (9). In a subsequent session, subjects were exposed to a mental arithmetic and noise stressor. The results revealed that the stressor decreased the blastogenic response of PBLs to concanavalin A (Con A) and increased NK cell percentages and NK cytotoxicity, absolute numbers of CD8⁺ T lymphocytes, NE and EPI levels, HR, and blood pressure responses. Importantly, not only did individuals classified during the prescreening as high-HR reactors demonstrate larger stress-related elevations in HR, but they also showed higher stress-related changes in plasma cortisol concentrations and NK cell cytotoxicity than individuals classified as low-HR reactors (9). Finally, Benschop et al. (15) examined the effects of β -adrenergic blockade (40 mg of propranolol) on the cardiovascular and immune responses to a brief psychological stressor. They found that the stressor increased HR, decreased preejection period (PEP), and increased the cytotoxicity and number of circulating NK cells and that β -blockade eliminated these cardiac and immune responses to the stressor. These studies suggest that HR reactivity may be a marker or outcome of a common mechanism triggered by an acute psychological stressor that contributes to the regulation of cellular immune responsiveness. The finding that cortisol concentration was heightened in high-HR reactors is particularly provocative in view of the extensive literature that links cortisol with the down-regulation of multiple aspects of cellular immune function.

When HR reactivity has been used to explore the heterogeneity in the response of subjects to psychological stressors, it has tended to be treated as a unidimensional (and, occasionally, as a unidirectional) construct that ranges from low to high, which reflects individual differences in adrenergic reactivity to daily stressors and behavioral challenges. Although adrenergic activity exerts predominant control over the vasculature and cardiac inotropy, cardiac chronotropy is a joint function of sympathetic and vagal activity. Moreover, psychological stressors are now known to affect vagal and sympathetic outflows to the heart (26–28), and these out-

flows can vary independently (20, 29, 30). An individual's classification as high in HR reactivity ignores possible individual differences in the autonomic origins of this reactivity. Such a classification could originate in elevated sympathetic reactivity, vagal withdrawal, or reciprocal activation of the sympathetic and vagal outflows to the heart. Conversely, an individual's classification as low in HR reactivity could stem from low sympathetic (and vagal) reactivity or from low to high coactivation of the sympathetic and vagal controls on cardiac chronotropy. Although these issues have long been recognized (31), research on cardiac reactivity has generally emphasized variations in HR reactivity per se rather than variations in the autonomic origins of HR reactivity. The classification of subjects in terms of HR reactivity relegates variations in the autonomic origins of HR reactivity to the error term, a practice that may obscure the relationship between autonomic responses to stressors and neuroendocrine, immune, or health outcomes. Research with autonomic blockades and noninvasive indices has demonstrated that cardiac PEP and respiratory sinus arrhythmia (RSA) can provide valid and reliable markers of cardiac sympathetic activation and cardiac vagal activation, respectively, in response to brief psychological stressors (32, 33). Therefore, we quantified interindividual variations in cardiac sympathetic reactivity (as indexed by PEP reactivity in our reactivity paradigm) and in cardiac vagal reactivity (as indexed by RSA reactivity) in addition to HR reactivity to explore the mechanisms that underlie the heterogeneity in human responses to brief psychological stressors.

METHOD

Subjects

Twenty-two older women (21 white and 1 African American), ranging in age from 56 to 73 years (mean_{age} = 66.9, standard error of the mean [SEM] = 0.93) served as subjects and were tested in the Clinical Research Center of the Ohio State University Hospital. The subjects were part of a longitudinal project but participated in this study over a 3-month period. Subjects were paid \$75.00 for 2.5 hours of participation in this study. The inclusion criteria for participation were that subjects a) were in good health and were normotensive; b) had no history of psychological disorder or chronic illness; c) were not receiving any cardiovascular prescription medication, nonprescription drugs, or tobacco products; d) exercised, on average, less than 10 hours per week; e) consumed, on average, less than 10 alcoholic beverages per week; and f) were not math, speech, or needle phobic. Subjects were asked to refrain from ingesting antiinflammatory agents, antihistamines, or alcohol during the 24 hours preceding the test day. All women were postmenopausal, and 27% were receiving estrogen

replacement therapy. Therefore, whether or not subjects were receiving estrogen therapy was treated as a between-subjects factor in the experimental design. Technical problems resulted in incomplete immune data from two to seven subjects, depending on the assay and incomplete impedance cardiograph data from three subjects. In addition, complete vaccine and cardiovascular data were available for a subset of 8 of the 22 subjects. The degrees of freedom were adjusted accordingly.

Procedure

In preparation for the study, subjects were asked a) to reschedule their appointment if they become ill or experienced a major negative life event (e.g., death in the family), b) not to consume any alcohol or take any nonprescription medication (e.g., antihistamines) the day before the study, c) to refrain from smoking and any exercise the day before the study, and d) to refrain from eating or drinking anything besides water from midnight to the time of their scheduled appointment. All subjects were tested at approximately the same time in the morning. When the subjects arrived, the tasks and measures were reviewed, the subjects' questions were answered, and informed consent was obtained. An occluding cuff of appropriate size was placed over the brachial artery of the left arm for continuous blood pressure measurements, and spot electrodes for impedance cardiography were attached.¹

In addition, an 18-gauge, indwelling catheter was inserted into an antecubital vein on the subject's arm. After venipuncture, subjects spent approximately 20 minutes completing an innocuous set of questionnaires. Among these scales was a health/lifestyle questionnaire that assessed their exercise habits, caffeine consumption, family history of hypertension, and number and nature of major negative life events during the last year. The subjects then rested in a supine position for 30 minutes to allow adaptation. After this adaptation period, the subjects were seated in an upright position and instructed to relax for 6 minutes. To assess baseline activity, cardiovascular and respiration measures were recorded during the final 5 minutes, and a 40-ml blood sample was collected at the end of this period for endocrine and immune assays.

After these baseline measures, the subjects received instructions for both tasks, and any questions about the tasks were answered. Because we were interested in the effects of brief psychological stressors representative of those individuals encounter in their daily lives rather than in the effects of a specific psychological stressor, the second stressor immediately followed completion of the first, and the order of these stressors was counterbalanced across subjects to allow examination of the separate effects of time and type of stressor. Cardiovascular and

¹ Because our primary interest in the cardiac assessments were the heart period time series (for calculation of RSA) and systolic time interval measures (particularly the PEP) and because spot electrodes are more convenient and comfortable for subjects, we used the spot electrode configuration. Sherwood et al. (35) compared impedance measures recorded with spot versus hand electrodes and reported comparable data and reliabilities for systolic time interval measurements. In addition, the reliability estimates for HR, PEP, RSA, HR reactivity, PEP reactivity, and RSA reactivity with spot electrodes are adequate for individual difference analyses (20).

respiration measures were collected throughout the 6-minute speech stressor period and throughout the 6-minute math stressor. A 20-ml blood sample was collected immediately after the first stressor for endocrine assays, and another 40-ml blood sample was collected immediately after the second stressor for endocrine and immune assays. Thus, a) cardiorespiratory activity was recorded during three periods: 5-minute baseline, 6-minute speech stressor, and 6-minute mental arithmetic stressor (with order of stressors counterbalanced across subjects); and b) three blood draws were taken: 40 ml immediately preceding the introduction of the stressors for endocrine and immune assays (baseline: time, 0 minutes), 20 ml immediately after the first stressor (midstressor: time, 6 minutes) for endocrine assays, and 40 ml immediately after the second stressor (poststressor: time, 12 minutes) for endocrine and immune assays.

Speech stressor. The speech stressor method of Saab et al. (13) was used. The subjects were asked to imagine that they were in a department store shopping when a security guard falsely accused them of shoplifting. They were instructed to prepare a 3-minute speech to a) tell their side of the story, b) tell the manager what the security guard did wrong and why the security guard may have suspected them of shoplifting, c) say how they can prove they did not steal the item, d) specify what should happen to the security guard for the mistake, and e) summarize their points. The subjects were instructed to give intelligent and well-thought out answers because their speech would be recorded and compared with the speeches of others. They were given 3 minutes to prepare and 3 minutes to present their speeches.

Math stressor. The subjects performed six 1-minute serial subtraction problems continuously for 6 minutes. They were instructed that any error they made would be corrected by the experimenter and that they should continue from the correct number. The minuend for minute 1 was 297; for minute 2, it was 688; for minute 3, it was 955; for minute 4, it was 593; for minute 5, it was 1200; and for minute 6, it was 1741. The results from our prior research on mental arithmetic in older adults indicated that subjects average approximately 10 serial subtractions per minute (34). The subtrahend in minute 1 was 3. To maintain maximal task involvement and moderate task difficulty (i.e., approximately 10 correct answers/minute), the subtrahend specified for each subsequent minute was contingent on the subject's performance during the preceding minute (Table 1). Two measures of task performance were calculated: the number attempted and the percentage correct. Given the design of this task to accommodate individual differences in mathematical proficiency in older adults, these performance measures were not expected to differ across groups.

Measures

A Minnesota Impedance Cardiograph (model 304B) (Sucom, Inc, Minneapolis, MN) was used to measure EKG, basal thoracic

Table 1. Subtrahend Specified as a Function of Performance on the N - 1 Problem

Minutes	Number Correct on N - 1 Problem					
	<6	6-15	16-25	26-35	36-45	>45
2	3	4	7	8	14	13
3	3	5	12	12	17	24
4	2	3	8	16	23	27
5	2	4	6	13	24	34
6	2	3	7	17	27	37

impedance (Z_0), and the first derivative of the impedance signal (dZ/dt). Disposable EKG spot electrodes were placed in the tetrapolar configuration. Although band electrodes provide more accurate magnitude measures of cardiac output values, PEP can be measured with equal accuracy with band or spot electrodes (35). The two outer (current) electrodes were placed over the fourth cervical vertebra and the ninth thoracic vertebra, whereas the two inner (recording) electrodes were placed 4 cm above the clavicle and over the sternum at the fourth rib. A 4-mA alternating current at 100 kHz was passed through the two outer electrodes, and Z_0 and dZ/dt were recorded from the two inner electrodes.

Blood pressure was recorded with the Cortronics 7000 blood pressure monitor (Cor Medical Corp, Kings Park, NY), and respiration were recorded with an EPM systems amplifier (EPM Systems, Midlothian, VA) and strain gauge placed below the lowest current electrode. The impedance cardiograph, blood pressure monitor, and respirometer were interfaced to a laboratory micro-computer, and the basal thoracic impedance (Z_0), the first derivative of the pulsatile impedance signal (dZ/dt), the EKG, systolic (SBP) and diastolic blood pressures (DBP), and respiratory activity were converted to digital signals (12-bit analog-digital converter, 500 Hz for dZ/dt and EKG; 250 Hz for Z_0 , 5-beat averages for blood pressure, and 250 Hz for respiration) and stored for off-line data reduction and analysis with an interactive program. To minimize artifacts in recording interbeat intervals, the EKG from the impedance cardiograph was bandpass filtered with cutoffs at 1 Hz and 10,000 Hz before digitization. In addition, the EKG and dZ/dt waveforms were monitored continuously with a dual-trace oscilloscope to minimize artifacts during data collection.

The impedance data were ensemble averaged within 1-minute epochs, and each waveform was verified or edited before analyses. The PEP was quantified as the time interval in milliseconds from the onset of the EKG Q-wave to the B-point of the dZ/dt wave. The mean PEP was calculated for each minute for each subject. These minute-by-minute means were averaged over the 5-minute baseline and each 6-minute stressor to increase reliability. Mean SBP and DBP were calculated similarly.

The interbeat intervals were checked and edited for artifacts by the detection algorithm of Berntson et al. (36). The interbeat intervals were subsequently verified by visual inspection. The mean HR was calculated for each minute for each subject. In addition, the interbeat interval data were converted to a time series of successive 500-ms samples. The magnitude of RSA was extracted as a noninvasive index of cardiac vagal activity with a personal computer-based software package (MXedit 2.01, Delta-Biometrics, Bethesda, MD). As suggested by Porges and Bohrer (37), the complex trend in the heart period time series was removed with a 21-point cubic digital filter that was moved stepwise through the data. The resulting smoothed time series was subtracted from the original time series to generate a residual series. The natural logarithm of the variance of the heart period pattern within the frequency bandpass associated with respiration (i.e., 0.12 to 0.40 Hz) was calculated for the estimate of cardiac vagal activity. Mean RSA activity was calculated across each 1-minute period for each subject. These minute-by-minute means were averaged over the 5-minute baseline and each 6-minute stressor to increase reliability.

The digitized respiratory signal was subjected to a 10-point boxcar filter. Our recording and filtering procedures were developed in pilot testing on the speech stressor. The respiration data were verified or edited to eliminate artifacts, and the mean respiration rate, respiration amplitude, and inspiration-fraction were calculated for each minute for each subject. These minute-

by-minute means were averaged over the baseline and over each 6-minute stressor to increase their reliabilities.

Assays for EPI, NE, ACTH, and cortisol were performed with plasma from the blood samples drawn at baseline, after the first stressor (i.e., 6 minutes after the onset of the stressors), and after the second of the two stressors (i.e., 12 minutes after the onset of the stressors). Plasma ACTH levels were assayed with immunoradiometric assays supplied by Nichols Institute (Capistrano, CA). This assay has an intra- and interassay coefficient of variation of less than 10% and the sensitivity is 1 pg/ml. Plasma cortisol levels were assayed with a fluorescent polarization technique (TDX-Abbott Laboratories, Chicago, IL). This assay has an intra- and interassay coefficient of variation of less than 10%. Plasma catecholamine concentrations (EPI and NE) levels were determined by high-performance liquid chromatography (HPLC) with a Waters system (Millipore, Waters Division, Marlborough, MA) with an electrochemical detector. The sensitivity of this HPLC system for EPI is 10 pg/ml and for NE, is 20 pg/ml. This assay has an intra- and interassay coefficient of variation for EPI of 15% and for NE of 9%.

To perform the immune assays, approximately 40 ml of blood treated with ethylenediaminetetraacetic acid to prevent clotting was obtained from each participant at baseline, after the first stressor (midstressor), and after the second stressor (poststressors). Mononuclear cells were separated with Hypaque-Ficoll (Sigma Chemical Co., St. Louis, MO) density gradients, washed two times with magnesium- and calcium-free buffer, counted in a Coulter counter (Hialeah, FL), and then used as described. Fresh cells were used to perform all the cellular assays, and variables in the assays were controlled by using the same lot of media, the same lot of fetal bovine serum or human pooled serum, the same lot of plastic tissue culture plates, and the same technicians.

Complete blood cell counts and differentials, performed by the Clinical Immunology Laboratory at the Ohio State University Hospital, were obtained on each blood sample. The percentages of CD3+ (T lymphocytes), two subsets of T lymphocytes (CD4+ and CD8+), and NK cells were also determined by flow cytometry with the appropriate monoclonal antibodies (Coulter) and the routine procedures in our laboratories (38). NK cells have been shown to be important in the body's defenses against malignant disease (39) and to have an important role in eliminating virus-infected target cells (40). The procedures used for NK cell lysis in this study have been described in detail elsewhere (41). In this assay, the NK cells are the only subset of the PBLs that lyse the target (tumor) cells. PBLs were prepared at 50:1, 25:1, and 12.5:1 effector-to-target cell ratios and were seeded in triplicate in 96-well microtiter plates (Costar Corp., Pleasanton, CA). Additional wells that contained only target cells (K562) in medium that contained 1% sodium dodecyl sulfate were used to determine spontaneous and maximal release of radioactivity, respectively. NK lysis values were standardized at the 25:1 effector/target ratio with a logistic regression (42).

The procedures used to assess the blastogenic response of PBLs to Con A and PHA are described by Kiecolt-Glaser et al. (38). Cells were prepared (5×10^6) in complete RPMI-1640 medium, treated with Con A and PHA, which can stimulate T-lymphocyte proliferation, and incubated for 48 hours. The concentrations for Con A and PHA used were 2.5, 5.0, 10.0, and 20.0 μg . All samples were run in triplicate, and counts per minute were determined by averaging the triplicates and reported as the logarithm transformed values of the counts per minute. Preliminary analyses indicated that the highest concentrations of Con A and PHA produced floor effects in cell proliferation; so the concentrations

of 2.5, 5.0, and 10.0 μg served as the three levels of the concentration factor.

We also assessed the nutritional status of the subjects to rule out changes in the immune response that were related to malnutrition. To assess nutritional status, serum albumin and serum transferrin levels were measured. The former marker reflects long-term malnutrition, whereas the latter is more sensitive to nutritional intake over the previous 2 weeks. If either marker was out of the normal range, then the subject's immunological data were excluded. None of these markers was out of the normal range.

RESULTS

Cell means for the effects of the psychological stressor on autonomic activity are summarized in Table 2.² A repeated-measures analysis of variance (ANOVA) confirmed that the psychological stressor produced a large increase in HR ($F(2,36) = 28.19$, $p < .0001$, $H-F_{\text{epsilon}} = .70$), which was maintained across the 12-minute stress period (Table 2). To examine the autonomic determinants of this HR reactivity, PEP and RSA were subjected to repeated-measures ANOVAs. The results confirmed that the stressor abbreviated PEP ($F(2,36) = 9.14$, $p < .001$, $H-F_{\text{epsilon}} = .99$) and diminished RSA ($F(2,38) = 18.06$, $p < .0001$, $H-F_{\text{epsilon}} = .88$), consistent with the hypothesis that psychological stressors evoked reciprocal cardiac sympathetic activation and cardiac vagal withdrawal. The stressor also elevated SBP ($F(2,38) = 13.22$, $p < .0001$, $H-F_{\text{epsilon}} = .90$) and DBP ($F(2,38) = 10.07$, $p < .001$, $H-F_{\text{epsilon}} = .82$) and dampened respiration amplitude ($F(2,40) = 7.84$, $p < .002$, $H-F_{\text{epsilon}} = .93$), whereas respiration period was not affected significantly by the stressor (Table 2).

The mean neuroendocrine responses to the psy-

² Preliminary analyses indicated that the order of the psychological stressors and the type of psychological stressor (mental arithmetic and speech stressor) produced few significant effects and none that qualified the results reported in the text. In addition, 6 of the 22 subjects were receiving estrogen replacements; so the analyses were conducted with estrogen replacement as a between-subjects factor. Only three main effects for estrogen replacement were significant. Older women undergoing estrogen replacement were characterized by lower plasma concentrations of EPI ($F(1,18) = 4.90$, $p < .05$); higher proliferative responses of peripheral blood lymphocytes to Con A ($F(1,20) = 10.04$, $p < .005$), and higher proliferative responses of peripheral blood lymphocytes to PHA ($F(1,20) = 8.37$, $p < .01$). No interaction test that involved estrogen replacement was statistically significant. Therefore, analyses reported in the text were conducted collapsing across the order, type of stressor, and estrogen-replacement factors.

AUTONOMIC, ENDOCRINE, AND IMMUNE RESPONSES TO ACUTE STRESS

Table 2. Mean (\pm SEM) Autonomic Response as a Function of Psychological Stressor

Measure	Baseline	Midstressor	Poststressor
HR*	68.01 \pm 2.01	79.00 \pm 2.23	80.44 \pm 2.63
PEP*	95.46 \pm 4.17	85.81 \pm 4.36	86.23 \pm 4.67
RSA*	5.01 \pm 0.29	4.31 \pm 0.24	4.21 \pm 0.23
SBP*	128.53 \pm 4.09	140.97 \pm 5.14	136.38 \pm 4.96
DBP*	78.36 \pm 2.64	85.01 \pm 2.88	83.11 \pm 2.79
Resp. amplitude	6.11 \pm 0.11	5.95 \pm 0.10	5.92 \pm 0.09
Resp. period	4.52 \pm 0.23	4.23 \pm 0.15	4.07 \pm 0.12

HR, heart rate in beats per minutes; PEP, preejection period in milliseconds; RSA, respiratory sinus arrhythmia in log units; SBP, systolic blood pressure in millimeters of mercury; DBP, diastolic blood pressure in millimeters of mercury; Resp. amplitude, respiration amplitude in arbitrary units; Resp. period, respiration period in seconds.

* *F* ratio for stressor, $p < .001$.

Table 3. Mean (\pm SEM) Neuroendocrine Response as a Function of Psychological Stressor

Measure	Baseline	Midstressor	Poststressor
EPI**	22.18 \pm 1.91	42.27 \pm 3.80	35.18 \pm 2.48
NE**	454.05 \pm 33.33	609.77 \pm 40.94	595.46 \pm 41.16
ACTH*	11.23 \pm 2.77	16.31 \pm 3.70	14.77 \pm 3.57
Cortisol	12.19 \pm 0.94	12.06 \pm 0.90	11.74 \pm 0.97

Plasma epinephrine, norepinephrine, and ACTH concentrations are expressed in picograms per milliliter; plasma cortisol concentrations are expressed in micrograms per deciliter.

* *F* ratio for stressor, $p < .01$.

** *F* ratio for stressor, $p < .001$.

chological stressor are summarized in Table 3. The acute psychological stressor increased plasma EPI ($F(2,42) = 23.63$, $p < .0001$, $H-F_{\text{epsilon}} = .71$), NE ($F(2,42) = 36.67$, $p < .0001$, $H-F_{\text{epsilon}} = 1.00$), and ACTH concentrations ($F(2,42) = 7.42$, $p < .01$, $H-F_{\text{epsilon}} = .57$) but appeared to have no effect on the plasma cortisol level ($F(2,42) = 1.18$, $p = \text{NS}$). Pairwise comparisons indicated that the neuroendocrine responses to the stressor occurred within the first 6 minutes and that the EPI response to the stressor was maximal midstressor (Table 3). Although the mean ACTH concentration was highest midstressor, the mean midstressor and poststressor levels did not differ significantly, and both were higher than the mean ACTH at baseline.

Analyses of T-lymphocyte and NK cell function revealed that a) cell proliferation to Con A decreased as a function of concentration ($F(2,42) = 133.05$, $p < .001$, $H-F_{\text{epsilon}} = .76$) and as a function of the psychological stressor ($F(1,21) = 4.56$, $p < .05$); b) the blastogenic response to PHA decreased as a function of concentration ($F(2,42) = 11.94$, $p < .01$, $H-F_{\text{epsilon}} = .67$) but did not decrease significantly as a function of psychological stressor ($F(1,21) = 2.26$, $p > .10$); and (c) NK cell cytotoxicity increased after

the psychological stressor ($F(1,21) = 19.26$, $p < .001$, Table 4). The brief psychological stressor also resulted in more circulating white blood cells ($F(1,15) = 9.97$, $p < .01$, mean_{baseline} = $5.241 \pm 0.151 \times 10^3$ cells/ m^3 , mean_{poststressor} = $6.200 \pm 0.239 \times 10^3$ cells/ m^3), lymphocytes ($F(1,15) = 11.43$, $p < .005$, mean_{baseline} = $1.432 \pm 0.055 \times 10^3$ cells/ m^3 , mean_{poststressor} = $1.906 \pm 0.140 \times 10^3$ cells/ m^3), and T lymphocytes ($F(1,12) = 28.31$, $p < .001$, mean_{baseline} = $0.987 \pm 0.051 \times 10^3$ cells/ m^3 , mean_{poststressor} = $1.216 \pm 0.827 \times 10^3$ cells/ m^3). Analyses of T-lymphocyte and NK cell percentages revealed that the psychological stressor decreased the percentage of helper/inducer cells (CD4+) ($F(1,14) = 20.52$, $p < .001$), increased the percentage of suppressor/cytotoxic (CD8+) cells ($F(1,14) = 5.00$, $p < .05$), reduced the ratio of circulating helper to suppressor/cytotoxic T cells (CD4+/CD8+) ($F(1,14) = 16.88$, $p < .001$), and increased the percentage of NK cells ($F(1,14) = 12.64$, $p < .005$, Table 5). Analyses of absolute cell numbers (lymphocytes) produced comparable results with the exception of the number of helper/inducer T-cells (CD4+), which was unaffected by the stressor.

Regression analyses (treating HR, PEP, and RSA reactivity indices as continuous rather than dichotomous individual difference variables) were performed next to examine the hypothesis that the sympathetic substrate of HR reactivity, as indexed by PEP reactivity rather than HR reactivity per se, would be more strongly related to stress-related neuroendocrine and immune changes.³ For instance, although nomothetic analyses indicated that cortisol concentrations were unaffected by the psychological stressor, idiographic analyses revealed considerable interindividual variation in response to the stressor. These interindividual variations in cortisol response were predicted well by cardiac sympathetic reactivity (as indexed by PEP reactivity; $r = -.62$, $t(17) = -3.25$, $p < .005$), i.e., individuals who were characterized by high cardiac sympathetic reactivity were also characterized by higher stress-related changes in plasma cortisol. Importantly, these changes in cortisol concentrations were not predicted signifi-

³ Llabre et al. (43) showed that simple change scores of cardiovascular reactivity to behavioral challenges have comparable or superior reliability and specificity to residualized change scores. Following Llabre et al.'s recommendations, we measured reactivity in the present research with simple change scores (e.g., (midstressor+poststressor)/2 - baseline). For purposes of completeness, however, we also repeated the analyses reported in the text with residualized change scores. The same pattern of data was found in these ancillary analyses.

Table 4. Mean (\pm SEM) Blastogenic Response and Natural Killer Cytotoxicity (Collapsed Across Concentration and Effector/Target Ratios) as a Function of Psychological Stressor

Measure	Baseline	Poststressor
Con A* (2.5 mg)	4.41 \pm 0.03	4.28 \pm 0.06
Con A* (5 mg)	4.28 \pm 0.04	3.95 \pm 0.20
Con A* (10 mg)	3.51 \pm 0.07	3.14 \pm 0.18
PHA* (2.5 mg)	4.57 \pm 0.03	4.26 \pm 0.21
PHA* (5 mg)	4.56 \pm 0.04	4.26 \pm 0.21
PHA* (10 mg)	4.51 \pm 0.04	4.21 \pm 0.21
NK cell cytotoxicity**	45.85 \pm 3.99	55.37 \pm 3.87

Con A and PHA are expressed as mean logarithms of the counts per minute values at each of three concentration levels, and NK cell cytotoxicity is expressed as percent lysis standardized to a logistic curve at the 25:1 effector-to-target ratio.

* *F* ratio for stressor, $p < .05$.

** *F* ratio for stressor, $p < .001$.

Table 5. Mean (\pm SEM) Percent Subpopulations of Leukocytes as a Function of Psychological Stressor

Measure	Baseline	Poststressor
CD4 ⁺ ***	47.39 \pm 2.20	39.85 \pm 2.63
CD8 ⁺ **	23.76 \pm 1.36	27.48 \pm 2.10
CD4 ⁺ /CD8 ⁺ ***	2.16 \pm 0.22	1.61 \pm 0.17
NK Cells**	12.64 \pm 1.80	20.61 \pm 3.51

CD4⁺, CD8⁺, and NK cell measures are percentages.

* *F* ratio for stressor, $p < .05$.

** *F* ratio for stressor, $p < .01$.

*** *F* ratio for stressor, $p < .001$.

cantly by individual differences in overall HR reactivity ($r = .31$, $t(17) = 1.35$, $p > .15$) or RSA reactivity ($r = .18$, $t(18) < 1$, $p = \text{NS}$). Interindividual variations in cortisol response to the stressor were also predicted by DBP reactivity ($r = .47$, $t(18) = 2.28$, $p < .05$) and the prediction by SBP reactivity approached statistical significance ($r = .44$, $t(18) = 2.05$, $p = .056$). Therefore, a multiple regression analysis was performed with PEP reactivity, SBP, and DBP reactivity as simultaneous predictor variables. The results indicated that the three variables predicted 40% of the variance in the stress-related cortisol changes ($F(3,14) = 3.29$, $p < .05$) and that PEP reactivity ($t = -2.12$, $p = .05$) but not SBP reactivity or DBP reactivity was uniquely related to cortisol changes (t values < 1). Not surprising, given the nonsignificant relationship between HR reactivity and cortisol changes, this same pattern of results was obtained when HR reactivity was also included as a fourth predictor in the multiple regression equation. Together, these data suggest that brief psychological stressors have an impact on the hypothalamic pituitary adrenocortical axis in some individuals, specifically, individuals who are characterized by high cardiac sympathetic reactivity to psychological stressors.

Similarly, interindividual variations in stress-induced changes in plasma ACTH concentrations were predicted significantly by PEP reactivity ($r = -.70$, $t(17) = -4.04$, $p < .001$) and HR reactivity ($r = .63$, $t(17) = 3.34$, $p < .004$) but not by RSA reactivity ($r = .09$, $t(18) < 1$, $p = \text{NS}$). In addition, ACTH changes were predicted by SBP reactivity ($r = .64$, $t(18) = 3.52$, $p < .003$) and DBP reactivity ($r = .69$, $t(18) = 4.03$, $p < .001$). A multiple regression analysis was performed with PEP reactivity, HR reactivity, SBP reactivity, and DBP reactivity as predictors. These four predictors accounted for 70% of the variance in stress-related changes in plasma ACTH levels ($F(4,14) = 8.28$, $p < .01$), but only PEP reactivity ($t = -1.67$) and DBP reactivity ($t = 1.65$) approached significance as individual predictors (HR reactivity and SBP reactivity, t values < 1).⁴

DISCUSSION

In the older women that we evaluated, the brief psychological stressors heightened autonomic activation (as indexed by tachycardia, a shortening of PEP, and a diminution of RSA), elevated sympathetic adrenomedullary activity (as indexed by increased plasma catecholamine concentrations), and affected the cellular immune response (as indexed by cell proliferation after treatment with Con A, NK cell percentages and cytotoxicity, circulating CD4⁺ and CD8⁺ cells, and the CD4⁺/CD8⁺ ratio). The finding that the brief stressors increased autonomic activity and plasma catecholamine levels was consistent with the results of a large body of literature on brief psychological stressors (8, 9, 12, 18, 44, 45). Inspection of Tables 2 and 3 reveals that these effects were manifest by midstressor and were maintained throughout the stressor period.

In addition, the most common immunological effects linked previously to brief psychological stressors, i.e., increased circulation of NK cells, increased

⁴ Although the psychological stressor had strong effects on cardiovascular and catecholaminergic responses (Tables 2 and 3), analyses indicated that the EPI and NE changes attributable to the stressor were moderately and nonsignificantly related to interindividual variations in PEP reactivity (r values = .24 and $-.37$, respectively; p values $> .12$), HR reactivity (r values = .02 and .12, respectively; p values $> .60$), or RSA reactivity (r values = .38 and $-.30$, respectively; p values $> .09$); and EPI and NE changes were moderately and nonsignificantly to SBP reactivity (r values = .13 and .21, respectively; p values $> .35$) or DBP (r values = .24 and .01, respectively; p values $> .30$).

NK cell lysis, and decreased blastogenic response to the mitogen Con A (see review, Ref. 2), were all replicated in our sample of older women. The additional cellular immune responses to the stressors found in this research (e.g., increased CD8⁺ cell numbers) also mirrored the results observed in the two prior studies most similar to the present study. Naliboff et al. (44) examined the effects of a 12-minute mental arithmetic task and control film on cardiovascular and immune response in 12 young and 12 older women, and Sgoutas-Emch et al. (9) examined the effects of a 12-minute mental arithmetic plus unavoidable noise stressor in 22 healthy undergraduate men who were characterized dispositionally as being high or low in HR reactivity. As in the present study, these authors found that brief psychological stressors increased cardiovascular activity, elevated plasma catecholamine levels, and increased the percentage of circulating suppressor/cytotoxic T (CD8⁺) cells and NK cells. Sgoutas-Emch et al. (9) also examined the blastogenic response to mitogens and, as in the present study, found that brief psychological stress diminished proliferation to Con A. The stressors elevated catecholamine levels and depressed the proliferative response of PBLs in nearly all of the subjects in the present study, in accordance with the notion that catecholamines are involved in the regulation of the proliferative response of T lymphocytes to mitogens. The effect of the brief psychological stressors also tended to be more apparent in circulating NK cells and T-suppressor/cytotoxic cells than for T-helper cells, consistent with the greater density and sensitivity of β -adrenergic receptors on the former cell types (46).

Also consistent with prior research (47), the brief psychological stressors elevated plasma ACTH levels but not plasma cortisol levels over the periods measured in the present study. It is possible that the measurement periods were better suited to detect the effects of the brief stressors on ACTH than cortisol levels. For instance, Gallagher et al. (48) reported that cortisol secretions began about 10 minutes after initiation of ACTH secretion. These data were from a single subject, however. In a study of two normal subjects, Krieger and Allen (49) reported correlations between ACTH and cortisol concentrations over the entire testing period, but they emphasized that they also observed extended periods (30–50 minutes) during which marked rises in ACTH concentrations occurred without associated, or with markedly diminished, increments in plasma cortisol concentrations.

In the present study, the nomothetic analyses masked considerable individual differences in terms

of the impact of the stressors on plasma cortisol levels. All subjects were tested in the mornings, during which time cortisol concentrations typically decline (49). Nevertheless, 41% of the subjects in this research showed increases in plasma cortisol levels after the stressor, and considerable variability existed in the remainder of the sample in terms of the magnitude of the decline in cortisol levels across the course of the stressor. Given the effects of the stressor on ACTH and the brief duration of the stressor, the interindividual variation in stress-related cortisol levels is likely to be attributable to small episodes of cortisol secretion rather than cortisol clearance. Regression analyses designed to examine the organization of these individual differences confirmed that the older women who were characterized by high cardiac sympathetic reactivity to brief psychological stressors also showed high stress-induced changes in plasma cortisol and ACTH concentrations, which suggests the relative activation of the hypothalamic pituitary adrenocortical system in these subjects.

Individuals who display high-HR reactivity have often been assumed to be characterized by high sympathetic reactivity across effector organs and high sympathetic adrenomedullary reactivity. In an exemplary study, Benschop et al. (15) examined the cardiovascular and immune response to two active coping tasks, each of which was performed for 10 minutes. One half of the subjects were given a β -blocker (propranolol 40 mg), and the remaining subjects received a placebo 1 hour before the stressors. The stressors increased heart rate, SBP, and DBP and decreased PEP in the placebo group, whereas the stressors affected only SBP and DBP in the propranolol group. In addition, the stressors increased NK cell numbers in circulation and NK cell activity in the placebo group; these effects were abolished in the propranolol group, which suggests that the increases in NK cell number and activity of NK cells in circulation are controlled by a β -adrenergic mechanism. The PEP data in the Benschop et al. (15) study were consistent with recent double-blockade data (32, 33), which indicates that PEP is a sensitive and specific measure of cardiac sympathetic activation at baseline and in response to brief psychological stressors. Furthermore, the implication of Benschop et al.'s (15) blockade data that cardiac sympathetic activation is related to cellular immune responses to brief psychological stressors is congruent with the present research.

Benschop et al. (15) interpreted their data as indicating a general activation of the sympathetic nervous system, but neither general autonomic activa-

tion nor neuroendocrine (e.g., catecholamine) activity was measured. In the present study, the psychological stressors produced both cardiac sympathetic activation and cardiac vagal withdrawal, with concomitant elevations in heart rate. Although our results may be limited to older white women, the results from this study nevertheless suggest caution in equating cardiac sympathetic activation with general autonomic or catecholaminergic activation, i.e., basal and stress-related levels of EPI or NE were correlated weakly and nonsignificantly with interindividual variations in PEP reactivity and were uncorrelated with HR and RSA reactivity. These nonsignificant correlations are not attributable to insensitive cardiac or endocrine measures. As noted earlier, the stressors produced statistically significant effects on EPI and NE levels and all three cardiac indices, and PEP reactivity was predictably related to stress-related changes in ACTH and cortisol. This dissociation between the effects of the brief stressors on catecholamine levels and cardiac sympathetic activity is congruent with psychophysiological research on individual response stereotypy (50). Prior research has documented covariations among HR, cortisol, and catecholamine responses to stressors, but the stressors in these prior studies have tended to be more intense or enduring than the brief psychological stressors used in the present research (51). Lovallo et al. (18), and Sgoutas-Emch et al. (9), in contrast, also used mild psychological challenges and, as in the present research, found that cardiac reactivity correlated more highly with cortisol reactivity than with NE reactivity. Thus, the nature, intensity, and duration of the stressors are factors that may be important in terms of the generalizability of the present results.

The present data also illustrate the value of idiographic and nomothetic analyses in research on responses to psychological stressors in humans. Although the elderly women generally showed a heightened sympathetic adrenomedullary response to the psychological stressors, only a subset of these individuals also showed strong engagement of the hypothalamic pituitary adrenocortical system, specifically, those individuals characterized by high cardiac sympathetic reactivity. This result would have been missed entirely had this research been limited to description and discussion of group mean differences in responsiveness to stressors (52). By an examination of differences among individuals, evidence emerged that suggested that cardiac sympathetic reactivity is a marker or a common outcome of a precursor of hypothalamic-pituitary-adrenocortical activation such as corticotropin-releasing hor-

monone (53). Possible neural and/or receptor mechanisms remain to be clarified (46).

The brief psychological stressors used in this research were selected based on their known effects on HR reactivity and its autonomic origins (20, 32). The tasks were also designed to ensure that effort and success were comparable across subjects. For instance, the serial subtraction task was designed such that each subject averaged 10 correct responses/minute. One nevertheless might ask what are the effects of the verbal requirements of the psychological stressors. A recent study by McCann et al. (54) examined this question. In that study, the cardiovascular, catecholamine, and cortisol responses to two cognitively demanding tasks that required verbalizations (Stroop task and serial addition) were contrasted with the responses to two cognitively nondemanding tasks with comparable verbal demands. Their results revealed that the cognitively demanding tasks produced higher HR, SBP, DBP, EPI, and cortisol levels than the cognitively nondemanding tasks, even though the verbal requirements for these tasks were comparable.

Finally, although aging is associated with an apparent down-regulation of β -adrenergic receptors (55, 56), diminution of RSA (57), and decline in HR reactivity (58), older adults continue to be able to show large and significant increases in cardiovascular and endocrine responses to brief psychological stressors. Lindheim et al. (59), for instance, examined the cardiovascular and endocrine responses to math and speech stressors in pre- and postmenopausal women (mean_{age} = 36.7 and 55.7 years, respectively), with the reactivity assessment performed in the early follicular phase of the menstrual cycle (days 4 to 9) in premenopausal women. In general accord with the present experimental results, they found that the stressors elevated HR, SBP, DBP, ACTH, cortisol, androstenedione, and NE in both pre- and postmenopausal women. The only significant differences found by Lindheim et al. (59) between pre- and postmenopausal women in their response to the stressors was that postmenopausal women showed larger elevations in SBP and smaller elevations in cortisol than premenopausal women (cf. Ref. 13). Similarly, Barnes et al. (60) compared the HR, mean arterial pressure (MAP), NE, and EPI responses of 10 healthy young and 10 healthy old men (mean_{age} = 26.6 and 68.5 years, respectively) to two brief psychological stressors (digit span test and serial subtraction task). They, too, found that the stressors elevated HR, MAP, NE, and EPI levels. The only responses to the stressors that differentiated the young and old groups were HR reactivity, which was

greater in the young than in the older subjects, and the NE response to the stressors, which was larger in the older men. In light of Garwood et al.'s (61) finding that individual response stereotypy increases with age, this study suggests that interindividual variations in cardiac sympathetic reactivity may be especially helpful in accounting for some of the heterogeneity in the stress responses and health outcomes of elderly people.

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