

The effects of an acute psychological stressor on cardiovascular, endocrine, and cellular immune response: A prospective study of individuals high and low in heart rate reactivity

SANDRA A. SGOUTAS-EMCH,^a JOHN T. CACIOPPO,^{b,c,d} BERT N. UCHINO,^b
WILLIAM MALARKEY,^{a,c,e,f} DENNIS PEARL,^{f,g}
JANICE K. KIECOLT-GLASER,^{b,c,h} AND RONALD GLASER^{a,c,e,f}

^a Department of Medical Microbiology and Immunology, Ohio State University, Columbus

^b Department of Psychology, Ohio State University, Columbus

^c Brain, Behavior, Immunity, and Health Program, Ohio State University, Columbus

^d Center for Cognitive Science, Ohio State University, Columbus

^e Department of Medicine, Ohio State University, Columbus

^f Comprehensive Cancer Center, Ohio State University, Columbus

^g Department of Statistics, Ohio State University, Columbus

^h Department of Psychiatry, Ohio State University, Columbus

Abstract

High and low reactors were preselected on the basis of their heart rate reactivity to a speech stressor in a prescreening session. In the main study, subjects were exposed to a mental arithmetic plus noise stressor. Cardiovascular activity was recorded during baseline and stressor, and blood was drawn prior to and following the stressor for endocrine and immune assays. Results revealed that the stressor decreased the blastogenic response to concanavalin A and increased natural killer cell numbers and cytotoxicity, absolute numbers of CD8⁺ T-lymphocytes, nor-epinephrine and epinephrine levels, heart rate, and blood pressure responses. In addition, cortisol and natural killer cell cytotoxicity responses to the stressor differentiated individuals high versus low in heart rate reactivity. These results suggest that the interactions among the autonomic nervous system, endocrine system, and immune system are not only amenable to psychophysiological analysis but that such analyses may play an important role in illuminating underlying mechanisms.

Descriptors: Heart rate, Psychological stressor, Individual differences, Cardiovascular reactivity, Endocrine response, Cellular immunity, Psychoneuroimmunology

Traditional views of the immune system have emphasized specific and nonspecific physiological responses to pathogens or tissue damage (Roitt, Brostoff, & Male, 1985). However, it is now clear that the immune system is influenced by central nervous

system processes that are shaped by psychological factors (see reviews by Ader, Cohen, & Felten, 1991, and Kennedy, Glaser, & Kiecolt-Glaser, 1990). It is becoming more apparent that an understanding of immunocompetence will be inadequate in the absence of considerations of psychological stressors and autonomic psychophysiology. Investigations of these interactions were stimulated by research demonstrating the direct and moderating effects of psychosocial factors (e.g., conditioned stimuli, bereavement, social support, major life events) on immune competence (e.g., see Kennedy et al., 1990) and were accelerated by research demonstrating that cardiovascular reactivity covaried with cellular immune response to psychological stressors (e.g., Manuck, Cohen, Rabin, Muldoon, & Bachen, 1991; see also review by Kiecolt-Glaser, Cacioppo, Malarkey, & Glaser, 1992). Thus, major advances in psychoneuroimmunology may derive from increasing the scope of the analysis to consider the contributions of autonomic and endocrinologic processes and mechanisms.

This study was partially supported by grants MH18831 and MH44660 from the National Institute of Mental Health, the General Clinical Research Center grant M01RR0034, the Ohio State University Comprehensive Cancer Center core grant from the NCI, CA16058, and grant DBS9211483 from the National Science Foundation.

We thank the nursing staff of the cardiovascular research center for drawing the blood samples and Sue Moseley, Julianne Dorne, Leigh Ann Kutz, Karen Brown, Hsiao-yin Mao, and Marco Vasquez for their expert technical assistance.

Address reprint requests to: Ronald Glaser, Ohio State University Medical Center, 218 Meiling Hall, 370 W. 9th St., Columbus, OH 43210-1238, or John T. Cacioppo, Department of Psychology, Ohio State University, Columbus, OH 43210-1222.

Chronic or long-term psychological stressors such as caregiving for a family member with Alzheimer's disease (Kiecolt-Glaser, Dura, Speicher, Trask, & Glaser, 1991), marital strife (Kiecolt-Glaser et al., 1987), and bereavement (Schleifer, Keller, Camerino, Thornton, & Stein, 1983) are associated with immunological down-regulation. Unlike these chronic stressors, transient psychological stress is a ubiquitous part of nearly everyone's daily life. The immunological consequences of brief psychological stressors have only recently been examined, however. Laboratory stressors such as unpredictable noise, mental arithmetic, public speaking, and the Stroop test provide a model for transient life stressors; these studies of brief psychological stressors have revealed changes in the numbers of circulating mononuclear cells as well as cell function (Bachen et al., 1992; Brosschot et al., 1992; Landmann et al., 1984; Manuck et al., 1991; Naliboff et al., 1991). Although the results from these studies are fairly consistent, conflicting evidence, particularly from studies observing decreases in the blastogenic response to the mitogens concanavalin (ConA) and phytohemagglutinin (PHA), have been reported (Bachen et al., 1992; Manuck et al., 1991; Knapp et al., 1992; Weiss et al., 1990; Zakowski, McAllister, Deal, & Baum, 1992). Thus, the first aim of the present study was to comprehensively examine the effects of a brief psychological stressor on multiple aspects of autonomic, neuroendocrine, and immune function.

We also investigated individual variation in immune and endocrine responses to acute psychological stressors. Studies of the immunological consequences of brief experimental stressors have provided preliminary evidence that individuals who exhibit relatively high cardiovascular/catecholamine reactivity (Bachen et al., 1992; Manuck et al., 1991) also exhibit larger immune changes. Manuck et al. (1991), for instance, were the first to demonstrate that individuals who showed relatively high, in contrast to low, cardiovascular and catecholaminergic reactivity to a brief psychological stressor also showed larger decreases in lymphocyte proliferation in response to PHA and larger increases in CD8 suppressor cell numbers. In a follow-up study, Bachén et al. (1992) again found decreases in lymphocyte proliferation in response to PHA but observed increased numbers of circulating natural killer (NK) cells and decreased numbers of CD4 lymphocytes rather than significant increases in the number of circulating CD8 lymphocytes; these changes were more apparent in individuals who showed the most pronounced sympathoadrenal activation on exposure to the laboratory stressor.

These studies are important because they bear on possible autonomic and endocrine mechanisms (e.g., sympathetic adrenomedullary system) underlying or contributing to the immunological changes due to psychological stressors. Consistent with the involvement of the sympathetic adrenomedullary system in immunological regulation, epinephrine infusions *in vivo* have consequences similar to those observed following acute psychological stress, including decreases in blastogenic responses to mitogens and increases in NK cell number and cytotoxicity (Crary et al., 1983). The pituitary adrenocortical system has also been shown to have immunoregulatory effects (Rupprecht et al., 1990-1991). Although the pituitary adrenocortical system is governed by hypothalamic mechanisms that can also affect autonomic activity (Sternberg, Chrousos, Wilder, & Gold, 1992), it is unclear whether cortisol is elevated by brief psychological stressors or covaries with cardiovascular reactivity. Lovallo, Pincumb, Brackett, and Wilson (1990) reported that high, but not

low, heart rate reactors showed significant elevations in plasma cortisol concentrations during aversive incentives, whereas Manuck et al. (1991) did not observe differences in cortisol response to their brief experimental stressor in either their low or high reactor groups. Therefore, a second aim of the present study was to measure catecholaminergic and cortisol responses to a brief psychological stressor.

Finally, the research by Manuck and his colleagues suggests that individuals characterized by high cardiovascular and/or catecholaminergic reactivity show exaggerated cellular immune response to acute stressors. As Manuck et al. (1991) noted,

These findings demonstrate that subjects differ substantially in their immunologic responsivity to stress, and that such differences parallel (and may be predicted by) interindividual variability on an index of concomitant sympatho-adrenal activation. Whether the latter characteristic reflects an enduring and broadly expressed dimension of individual differences in sympathetic reactivity . . . or a variability of response limited to the specific stressor presented in this investigation cannot be determined from the present data. (p. 113)

To address this issue, we determined individuals' heart rate reactivity to a psychological stressor in a prescreening study. Subjects who were characterized by high or low heart rate reactivity participated in a follow-up study in which cardiovascular, neuroendocrine, and immune responses to a different psychological stressor were monitored.

In light of the data demonstrating that catecholamine infusions decrease blastogenic responses to mitogens and increases NK cell number and cytotoxicity (Crary et al., 1983), we departed from Manuck and his colleagues' procedure of using catecholaminergic activation to identify high and low reactors. Instead, we examined whether interindividual variability on heart rate reactivity would predict differences in endocrinological and immunological response. Heart rate reactivity was selected primarily for two reasons. First, stable and generalizable individual differences in heart rate reactivity are identifiable when the reactivity assessment is based on multiple measures of heart rate during baseline and stressor periods (e.g., Kamarck et al., 1992; Kasprovicz, Manuck, Malkoff, & Krantz, 1990; Sherwood, Dolan, & Light, 1990; Turner, 1989). Second, results reported by Knapp et al. (1992) suggest that heart rate reactivity *per se* parallels interindividual variability in immune response to a brief psychological stressor. Knapp et al. (1992) examined autonomic and immunological responses while subjects recalled and relived maximally disturbing and maximally pleasurable emotional experiences. Correlational analyses between changes in autonomic and immunological variables during the negative emotion revealed that heart rate correlated negatively and significantly with blastogenic responses to a mitogen (PHA) and correlated positively and significantly with NK cell numbers and total lymphocyte cell numbers. These correlational data suggest that heart rate reactivity may be a marker, or an outcome, of a common mechanism triggered by an acute psychological stressor that contributes to the regulation of cellular immune responsivity.

Method

Subjects

Forty-four undergraduate healthy men ranging in age from 18 to 31 years participated in a prescreening study in which heart rate (HR) reactivity to a brief speech stressor was assessed. Sub-

were isolated by density gradient centrifugation on Ficoll-Metrazoate gradients from 40 cc of heparinized venous blood, washed with complete RPMI 1640 medium, counted, and then prepared for the following assays.

NK cell activity (lysis). The procedures used for NK cell lysis in this study have been described in detail elsewhere (Glaser, Rice, Speicher, Stout, & Kiecolt-Glaser, 1986). Cells were prepared at 100:1, 50:1, 12.5:1, 6.25:1, and 3.12:1 effector to target cell ratios and were seeded in triplicate in 96-well microtiter plates (Costar Corp.). Additional wells containing only target cells (K562) in medium containing 1% sodium dodecyl sulfate were used to determine spontaneous and maximum release of radioactivity, respectively. NK lysis values were standardized at the 25:1 effector/target ratio using a logistic regression (Kazimer, Whisler, Stephens, Pearl, & Yates, 1989).

NK, CD4⁺, and CD8⁺ cell counts. The percentage of NK, CD4⁺, and CD8⁺ cells were determined by flow cytometry using the monoclonal antibodies T4, T8, and NKH-1 (Coulter), respectively, and using routine procedures in our laboratories (Kiecolt-Glaser et al., 1991). Cell culture supernatants were assayed for lymphokines using commercially available kits (Genzyme, Inc., Cambridge, MA). Absolute numbers were calculated by using the percentage of these cells and the absolute number of lymphocytes taken from the CBCs.

Blastogenic response to ConA and PHA. The procedures used in this study have been described (Kiecolt-Glaser et al., 1991). Cells were prepared (5×10^6) in complete RPMI 1640 medium, treated with ConA or PHA, and incubated for 48 hr. The concentrations for ConA and PHA used were 2.5, 5.0, 10.0, and 20.0 mg. 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. Because of technical problems, the blastogenic responses to PHA at the 5.0 mg concentration were not assayed.

The nutritional status of the subjects must be monitored to rule out changes in the immune response that are related to malnutrition. Therefore, we measured serum albumin at baseline, as described by Kiecolt-Glaser et al. (1991). If this marker had been out of the normal range, then the subject's immunological data for that sample would have been excluded. All of the subjects' data fell within the normal range.

Results

Prescreening Study

We first examined the internal consistency of HR, systolic blood pressure (SBP), and diastolic blood pressure (DBP). The two periods included in the analyses were baseline and speech periods. The Cronbach alphas for the measure of HR, SBP, and DBP were .80, .73, and .72, respectively. To examine whether the effects of the speech stressor in our prescreening replicated results of prior research, we conducted a repeated measures analysis of variance (ANOVA) on the HR and blood pressure data. As expected, the analyses indicated that the speech stressor elevated heart rate ($M_{\text{baseline}} = 70.31$, $M_{\text{stressor}} = 88.22$), $F(1,43) = 127.07$ ($p < .001$), SBP ($M_{\text{baseline}} = 128.11$, $M_{\text{stressor}} = 132.95$), $F(1,41) = 8.65$ ($p < .01$), and DBP ($M_{\text{baseline}} = 72.57$, $M_{\text{stressor}} = 78.77$), $F(1,41) = 27.56$ ($p < .001$).

Our aims in this study were to extend prior research by examining a variety of endocrine and immune responses to a brief psychological stressor and to examine whether individuals who differed in HR reactivity showed differential endocrine and immune responses to the stressor. Therefore, we identified the 12 subjects who showed the highest HR reactivity and the 12 subjects who showed the lowest HR reactivity to the speech stressor. These 24 subjects were then recruited to participate in the follow-up study. One subject declined participation and one subject failed to show up for his scheduled experiment time, resulting in a sample of 11 high HR reactors and 11 low HR reactors.

Subjects were assigned to the low or high HR reactor group based on their reactivity to the speech stressor; the mean change of low reactors was 5.32 bpm, and the mean change of high reactors was 30.08 bpm. The repeated measures ANOVA using these 22 subjects showed that the speech stressor elevated heart rate, $F(1,20) = 161.80$, $p < .001$. This analysis (a 2×2 ANOVA) does not necessarily mean that low and high HR reactors had comparable HR at baseline, however. To assess this, a between-subjects ANOVA was performed on mean HR during baseline. Results indicated high and low HR reactors had comparable basal HR ($M_{\text{low reactors}} = 71.64$, $M_{\text{high reactors}} = 72.62$), $F(1,20) < 1$.²

To examine potential confoundings with our blocking variable of HR reactivity, we performed several one-way ANOVAs with HR reactivity (low vs. high) as a between-subjects variable. The major dependent measures were (a) average hours of exercise per week, (b) average number of times exercising per week, (c) average number of alcoholic beverages per week, (d) height, (e) weight, (f) hours of sleep the prior night, (g) hours of vigorous activity during the past week, (h) alcohol consumption in the prior 48 hr, and (i) caffeine consumption over the prior 48 hr. Analyses revealed no significant effects. Thus, the blocking variable of HR reactivity did not appear to be confounded with the lifestyle variables measured in this study. Nutritional status, assessed by plasma albumin levels (Kiecolt-Glaser et al., 1991) in the follow-up session, showed no group difference. Hence, these measures are not discussed further.

Main Study

The cardiovascular measures from the main study were first subjected to 2 (group: low vs. high HR reactors) \times 2 (period: baseline vs. math stressor) ANOVAs to examine whether the high HR reactors responded more strongly to the mental arithmetic stressor than did the low HR reactors (Table 1). Main effects for period indicated that the experimental stressor elevated HR, $F(1,20) = 82.41$, $p < .001$, and DBP, $F(1,20) = 7.20$, $p < .02$. The mental arithmetic stressor led to significant Group \times Period interactions for HR, $F(1,20) = 11.15$, $p < .01$, SBP, $F(1,20) =$

²The selection of the top and bottom most reactive men in terms of HR reactivity does not guarantee that pressor levels and responses to the speech stressor were statistically comparable across the low and high reactor groups. Therefore, we repeated the analyses but this time limited the subjects in the analyses to the 22 subjects who participated in the main study. Results revealed that the speech stressor nonsignificantly increased SBP ($M_{\text{baseline}} = 128.76$, $M_{\text{stressor}} = 132.11$), $F(1,19) = 2.84$, $p < .11$, and significantly increased DBP ($M_{\text{baseline}} = 71.89$, $M_{\text{stressor}} = 76.82$), $F(1,19) = 8.76$, $p < .01$. No other main effect or interaction reached statistical significance. Thus, the high and low HR reactors differed in HR reactivity but were comparable in terms of the pressor reactivity evoked by the brief speech stressor.

to ConA revealed a significant main effect for concentration, $F(3,57) = 547.79, p < .001$, reflecting decreased cell proliferation as concentration increased; a main effect for period, $F(1,19) = 6.53, p < .02$, indicating that the stressor led to a small but significant decrease in the blastogenic response to ConA; and a Period \times Concentration interaction, $F(3,57) = 2.98, p < .03$, showing that the decreased blastogenic response to ConA following the stressor was more evident at high than low mitogen concentrations. This interaction is of the same form as the interaction observed previously for this measure in research on chronic stress (e.g., Kiecolt-Glaser et al., 1991). Analyses of the blastogenic response to PHA revealed only a main effect for concentration, $F(2,40) = 30.35, p < .001$.

Analyses contrasting high and low HR reactors' endocrine and immune responses to stressors uncovered a different pattern in the data. For instance, the stressor elevated the catecholaminergic response comparably in high and low HR reactors, but a significant Group \times Period interaction for cortisol, $F(1,20) = 5.92, p < .02$, revealed that high in contrast to low HR reactors showed larger stress-related increases in cortisol (see Table 2). Analyses also indicated that the high HR reactors showed larger stress-related increases in NK cytotoxicity, $F(1,17) = 7.29, p < .01$. Ancillary analyses confirmed that the HR reactivity measured in the prescreening session was positively and significantly correlated with both cortisol ($r = .51$) and NK cytotoxicity ($r = .52$). These data suggest that the brief experimental stressor affected cardiovascular, neuroendocrine, and immune response, and that individuals high in HR reactivity showed magnified cortisol and NK cytotoxicity responses to the stressor.⁴

Discussion

Recent research suggests that brief psychological stressors affect endocrine and cellular immune responses and that individual differences in cardiovascular reactivity are related to a subset of these responses. The present research was designed to address three limitations in this literature. First, prior research has relied on correlational analyses to examine individual differences in endocrine and immune responses to the brief experimental stressors. We used a short-term prospective design. We also used different psychological stressors to classify individuals in the prescreening study and to examine individual differences in stress-induced endocrine and cellular immune responses in the follow-up experiment. Together, these features of the study increase the likelihood that the classification of individuals in terms of their HR reactivity reflected a stable and generalizable individual difference. Second, individuals have tended to be classified as high or low reactors based on aggregate measures

of their cardiovascular and/or catecholaminergic reactivity to the experimental stressor, although significant correlations between HR and immune response have been reported (e.g., Knapp et al., 1992). To begin to identify what in this aggregate measure allows prediction of endocrine and immune responses to a psychological stressor, we preselected subjects who were characterized by high or low HR reactivity to a speech stressor in the prescreening session. Catecholamines per se can influence cellular immune function (Crary et al., 1983), so by not using catecholaminergic changes to define high and low reactors we could examine the predictive value of individual differences in autonomic (specifically, HR) reactivity in endocrinological and immunological response to acute psychological stress. Third, the endocrine and immune assays reported in prior research have been limited, so we performed a wider range of endocrine and immune assays (a) to better examine the specificity of the effect of psychological stressors on endocrine and immune function and (b) to determine which subset of the endocrine and immune responses to the experimental stressor differentiated individuals high versus low in HR reactivity.

The observed immunological and endocrinological changes replicated some of the prior effects that have been reported to result from acute stress, including decreases in lymphocyte proliferation in response to ConA (Knapp et al., 1992; Weiss et al., 1990; Zakowski et al., 1992), and increases in suppressor cell numbers (Brosschot et al., 1992; Manuck et al., 1991) and NK cell numbers (Bachen et al., 1992; Brosschot et al., 1992). The prior study most similar to our own is the investigation by Naliboff et al. (1991), who examined the effects of a 12-min mental arithmetic task and a control film on cardiovascular and immune response in 12 young and 12 elderly women. In the present study, we examined the effects of a 12-min mental arithmetic plus unavoidable noise stressor in 22 healthy undergraduate men, half of whom were characterized as high in HR reactivity and half of whom were characterized dispositionally as low in HR reactivity. Despite the differences in subject populations, both studies revealed that the brief stressor increased the number of circulating suppressor/cytotoxic T (CD8⁺) cells and the number of circulating NK cells. Furthermore, the brief stressor increased NK cytotoxicity in our study of young men, a result that was also observed in the young, but not in the elderly, women tested by Naliboff et al. (1991). We also found the stressor to increase circulating catecholamines, consistent with the observations of Naliboff et al. (1991), Dimsdale, Young, Moore, and Strauss (1987), Lovallo et al. (1990), Oleshansky and Meyerhoff (1992), and Manuck and his colleagues (e.g., Manuck et al., 1991). Within our sample of young men, the effects of the stressor were slightly greater for norepinephrine than epinephrine, with the blood pressure varying accordingly (i.e., stronger effects for DBP than SBP). Thus, the present results on the cardiovascular, neuroendocrine, and immunological consequences of an acute psychological stressor are generally consistent with those of past research, particularly those of studies using similar psychological stressors.

However, this is the first study to examine prospectively whether HR reactivity predicted stress-induced immunological and endocrinological changes. Although our use of male subjects leaves open the question of generalizability, our findings add to the extant literature in at least two ways: (a) high HR reactors showed a significant increase in cortisol, as compared with low HR reactors, following their exposure to the laboratory stressor (see, also, Lovallo et al., 1990), and (b) stress-

⁴The choice of metric (numbers, percentages) in the analyses of the immune data did not affect the pattern of statistical significance reported in the text. In addition, we repeated the analyses reported in the text using the HR reactivity measured in the main study rather than in the prescreening study. This was done to make it easier to compare our results with those that are already in the literature, in which concomitant analyses have been employed. All of the effects for period were replicated, and no new effects were statistically significant. Also, the statistically significant correlations between HR reactivity and changes in SBP ($r = .53, p < .02$), DBP ($r = .54, p < .01$), cortisol concentrations ($r = .52, p < .02$), and NK cytotoxicity ($r = .57, p < .01$) were replicated in the concomitant analysis.

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(RECEIVED March 29, 1993; ACCEPTED June 29, 1993)