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Persistent Alterations of the Autonomic Nervous System after Noncardiac Surgery

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Background: **Changes in the sympathetic nervous system may be a cause of postoperative cardiovascular complications. The authors hypothesized that changes in both β-adrenergic receptor (βAR) function (as assessed in lymphocytes) and in sympathetic activity (assessed by plasma catecholamines and by heart rate variability [HRV] measurements obtained from Holter recordings) occur after operation.**

Methods: **The HRV parameters were measured in 28 patients having thoracotomy (n = 14) or laparotomy (n = 14) before and for as long as 6 days after operation. Transthoracic echocardiography was performed before and on postoperative day 2. Lymphocytes were also isolated from blood obtained before anesthesia and again on postoperative days 1, 2, 3, and 5 (or 6). They were used to examine βAR number (B_{max}) and cyclic adenosine monophosphate (cAMP) production after stimulation with isoproterenol and prostaglandin E1. In addition, plasma epinephrine, norepinephrine, and cortisol concentrations were determined at similar intervals.**

Results: **After abdominal and thoracic surgery, most time and all frequency indices of HRV decreased significantly, as did B_{max} and basal and isoproterenol-stimulated cAMP production. The decrements in HRV correlated with those of B_{max} and isoproterenol-stimulated cAMP throughout the first postoperative week and inversely correlated with the increase in heart rate. Plasma catecholamine concentrations did not change significantly from baseline values, but plasma cortisol levels did increase after operation in both groups. Left ventricular ejection fraction was normal in both groups and unaffected by surgery.**

Conclusions: **Persistent downregulation and desensitization of the lymphocyte βAR/adenylyl cyclase system correlated with decrements in time and frequency domain indices of HRV throughout the first week after major abdominal or thoracic surgery. These physiologic alterations suggest the continued presence of adaptive autonomic regulatory mechanisms and may explain why the at-risk period after major surgery appears to be about 1 week or more. (Key words: β-adrenergic receptors; catecholamines; echocardiography; heart rate variability; stress response.)**

IN some patients undergoing noncardiac surgery, important cardiovascular events such as myocardial ischemia[1] or dysrhythmias[2] occur, most commonly on postoperative days 1-5. These early perioperative events have been associated with poor outcome at 18-24 months.[3,4] Sympathetic neural or hormonal mechanisms have been implicated as causative factors for these complications. In the study of possible effects of surgical stress on these mechanisms, the ability to assess autonomic outflow to target organs such as the heart would be important.

We chose to study the relation of sequential changes in time and frequency domain indices of heart rate variability (HRV), lymphocyte β-adrenergic receptor (βAR) function, and changes in hormones related to stress

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response during the first week after major thoracic or abdominal surgery. Analysis of HRV has been used extensively to investigate the modulation of autonomic influences on the heart in persons not undergoing surgery[5-9] and after cardiac[10] or noncardiac surgery[11,12]; however, the meaning of changes in HRV in response to surgery and postoperative events is not fully understood. Previously we showed that βARs of human lymphocytes, which have similar properties to those of the β_2-adrenoceptor system present in human right atrium and elsewhere,[13] are altered 1 day after operation.[14,15] We tested the hypothesis that alterations in the βAR system persist beyond the first day after surgery. Further, we determined plasma epinephrine, norepinephrine, and cortisol levels at intervals in an attempt to better understand whether they influence βAR and HRV changes during the first week after surgical stress.

Materials and Methods

The study was approved by the institutional review board of Memorial Sloan-Kettering Cancer Center, and informed consent was obtained from all patients. Two groups of patients having elective surgery for cancer were recruited; one consisted of 14 patients scheduled to undergo pulmonary lobectomy (thoracic group), and the other of 14 patients scheduled for major abdominal surgery (abdominal group). Excluded from the study were patients with a prior history of myocardial infarction, congestive heart failure, severe diabetes mellitus, or bronchospastic disease. No patient was receiving β-adrenergic agonists or antagonists, α-agonists or antagonists, calcium channel blockers, or corticosteroids. In the thoracic group, lobectomy was performed in 10 patients, pneumonectomy in 2 patients, and segmentectomy in 2 patients. The abdominal group included patients having hepatic resection (n = 4), pancreatic resection (n = 2), splenectomy combined with hepatic resection or thoracoscopy (n = 2), radical cystectomy (n = 1), radical nephrectomy (n = 1), and gastrojejunostomy and biliary bypass (n = 4).

All patients were premedicated with midazolam and glycopyrrolate 60-90 min before surgery. A thoracic epidural catheter was inserted before operation in 8 of 14 patients in the thoracic group, but no epidural local anesthetic was used. After an induction with thiopental and fentanyl, all patients received standard doses of isoflurane in oxygen and nitrous oxide supplemented with intravenous fentanyl and morphine as needed. All patients were extubated at the end of the operation and remained extubated through the postoperative course. Forty-five minutes before the expected end of surgery, 100-200 μg fentanyl in 10 ml saline was injected *via* the epidural catheter in eight patients. Epidural fentanyl was continued as needed in the postanesthesia care unit for 72-96 h. All other patients received postoperative analgesia with morphine sulfate *via* a patient-controlled analgesia pump for 72-96 h.

Ambulatory Electrocardiography and Heart Rate Variability Analysis

Dual-lead electrocardiogram recordings (leads CM_2 and CM_1 or CM_5) were made on Marquette 8500 Holter recorders (Milwaukee, WI). These recorders contain a digital clock to provide a time signal that is continuously recorded on the tape. One-hour recordings were made before premedication (preop baseline) and then continuously beginning on arrival in the postanesthesia care unit for 72 h. Another 1-h recording was obtained on postoperative day (POD) 5 or 6 if the patient was still hospitalized. The Holter tapes were digitized on a Marquette series 8000 scanner. The signal was sampled at 128 Hz. Sampling was triggered by the timing track on the tape to correct for flutter and wow of the recording or playback tape transport. QRS complex recognition and arrhythmia detection were done automatically by template matching. This system generates a beat-by-beat annotation of the electrocardiogram with a consistent and accurate time stamp for each QRS complex and classifies each complex as normal sinus, atrial or ventricular premature complex, or noise. The decisions made automatically by the computer were reviewed and corrected by an experienced technician and then by a cardiologist. When the HRV parameters were calculated, only normal-to-normal intervals were used. Each interval that was to be excluded because of ectopic beats or artifacts was replaced by holding the previous coupling interval level throughout the time interval to the next valid coupling interval. HRV indices measured were mean RR interval (ms) and its standard deviation (SDNN; ms); the standard deviation of 5-min mean RRs (SDANN; ms); the mean of all 5-min standard deviations of RRs (SDSD; ms); the root mean square of the difference of successive RRs (rMSSD; ms); the proportion of adjacent RRs >50 ms different (pNN50; %); total (TF; 0.01-1.00 Hz), low (LF; 0.04-0.15 Hz), and high frequency (HF; 0.15-0.40 Hz) power (ms^2). The LF:HF ratio has been proposed as an index of sympathovagal balance.[8] Fast Fourier transformation was used to com-

pute the power within the defined frequency limits for each 2-min interval (256 samples). For definitions of all HRV parameters, see the appendix.

Humoral and Lymphocyte β-Adrenergic Receptor Analysis

Blood samples (40 ml) for determination of lymphocyte βAR/adenylyl cyclase properties, lymphocyte subpopulation distribution, and plasma catecholamine and cortisol concentrations were obtained at 8:00–10:00 A.M. before induction (preop) and on the morning of POD1, POD2, POD3, and, if patients were still hospitalized, on POD5 or POD6. Blood samples from all patients were obtained from a vein before surgery and, in most cases, from a radial artery cannula on POD1. The remaining blood samples were obtained by venipuncture. No patient received exogenous adrenergic medications or glucocorticoids during the perioperative period. All blood samples were placed on ice immediately after collection. Samples for catecholamine and cortisol determination were centrifuged and the plasma stored at −70°C until analysis. Epinephrine and norepinephrine were analyzed by radioenzymatic assay, as previously described[16]; the limit of detection for epinephrine and norepinephrine were 10 pg/ml and 50 pg/ml, respectively. The intra- and interassay variations, respectively, were 6.2% and 8.0% for epinephrine and 6.4% and 13.3% for norepinephrine. Plasma cortisol concentration was measured using the Immuno-1 System (Bayer Corp., Tarrytown, NY) by magnetic separation competitive immunoassay using alkaline phosphatase as the conjugate marker. The limit of detection was 0.2 μg/dl; within-run variation at concentrations of 2.8, 16.8, and 30.6 μg/dl were 2.6%, 1%, and 1%, respectively; and the interassay variation at the same concentrations were 8%, 4.1%, and 3.9%, respectively.

Studies of βAR binding and cyclic adenosine monophosphate (cAMP) accumulation were performed using freshly prepared cells, usually within 5 h of blood collection. Lymphocytes were isolated from obtained whole blood by our previously described[14,15] modification of the method of Boyum,[17] with all manipulations done at 4°C to prevent alterations in the receptor status of the cells during isolation.[18] For studies of βAR binding, lymphocytes were suspended in Dulbecco's Modified Eagle Medium (Gibco Laboratories, Grand Island, NY) containing 20 mM HEPES and 0.1% bovine serum albumin adjusted to *p*H 7.4 with HCl (DMEH); for studies of cAMP accumulation, the cells were suspended in phosphate-buffered saline (10 mM Na_2HPO_4, 128 mM NaCl, 5.1 mM KCl, 1.2 mM $MgSO_4$, 5.0 mM glucose, adjusted to *p*H 7.4 with HCl). A portion of the DMEH suspension was used to determine the lymphocyte subpopulation distribution by indirect immunofluorescent flow cytometry[19] to ensure that any alterations detected in the adrenergic receptor and response system were not artifacts of alterations in subpopulation ratios.

Lymphocytes, 2.5×10^5 cells in 0.5 ml phosphate-buffered saline, were incubated for 10 min at 37°C in the presence of 1.0 mM 3-isobutyl-1-methylxanthine. Cyclic adenosine monophosphate production in the untreated state and in the presence of maximally stimulating concentrations of isoproterenol (10 μM) or prostaglandin E_1 (PGE_1; 10 μM) was assayed in triplicate, as previously described.[14,15] Accumulation of cAMP was measured by radioimmunoassay (Biomedical Technologies, Stoughton, MA).

Saturation binding curves (8–10 points) were determined in duplicate or triplicate with 3×10^5 cells in a final volume of 0.3 ml DMEH shaken gently at 4°C for 24 h in the presence of 2.5–240 pM [^{125}I]-iodopindolol (New England Nuclear Products, Boston, MA), specific activity 2200 Ci/mmol. Nonspecific binding was determined in the presence of 10 μM propranolol. After 24 h, the incubation was terminated by adding 4 ml of 1:10 phosphate-buffered saline (phosphate-buffered saline diluted 1:10 with water), and, to reduce nonspecific binding,[20] kept on ice for 5 min before filtration through Whatman GF/B filters (Gaithersburg, MD). The filters were washed three times with 5 ml phosphate-buffered saline and counted in a gamma counter at 75% efficiency. Specific binding averaged 73% and 68% of total binding at 2.5 and 240 pM, respectively. The βAR number (B_{max}) and apparent dissociation constant (K_D) were assessed by Scatchard analysis.[21]

Echocardiography

Transthoracic echocardiograms were performed before operation with the patient awake and supine using a 2.5/2.0-MHz transducer for imaging and Doppler echocardiography (SONOS 1500; Hewlett Packard, Waltham, MA). Subsequent echocardiograms were performed on all patients on POD2 or POD3. All patients were in sinus rhythm when echocardiography was performed. Any evidence of pericardial disease or changes in ventricular size were recorded. Left ventricular ejection fraction was measured by the established method of Teicholz from M-mode parasternal views. Because of technical difficulty, this measurement could not be made in two patients from each group after operation.

Data were later analyzed from videotape by one investigator (N.R.) blinded to the patients' clinical course.

Statistical Analysis

A power analysis was done to evaluate the ability to detect a ≥25% reduction from baseline B_{max} and isoproterenol-stimulated cAMP values (mean ± SD) on POD 1 using our previously published data.[15] We estimated that a sample size of 14 patients per group would be adequate to give a two-tailed paired analysis with 95% confidence intervals with power >90%. Values for cAMP generation, receptor number, binding affinity, lymphocyte subpopulation distribution, and catecholamine and cortisol concentrations within each group were compared using Student's paired *t* test, with Bonferroni correction for multiple comparisons. In some patients, the concentration of catecholamines or cortisol present was less than the limit of detection. In these cases, the value corresponding to the limit of detection was used for statistical analysis. The significance of difference between the thoracic and abdominal groups was assessed using the unpaired *t* test. Time and frequency domain parameters were analyzed using a repeated measures analysis of variance with contrasts.[22] The model is fitted using the method of restricted maximum likelihood using PROC MIXED in the SAS (version 6.0) statistical program (SAS Institute, Cary, NC). To examine the effects of anesthesia and surgery on HRV, preoperative (1 h) values of HRV were compared in two separate analyses with continuous 24-h data and with 1-h data (TF, LF, HF, and LF/HF) obtained from the corresponding hour of day from which preoperative values were made. The dependency of observation made within each patient over time was estimated using different models, and the one yielding the largest likelihood was chosen. The covariates considered were the pre- and postoperative times, type of operation, and any interaction between these covariates. Before analysis, TF, LF, HF, LF/HF, pNN50, and rMSSD were log-transformed. To examine the correlation of changes in heart rate, B_{max}, isoproterenol-stimulated cAMP and those in the frequency domain parameters of HRV, a repeated measures analysis of variance was done. The significance and direction of the correlation was established using the model coefficient. All data are reported as mean ± SD, with probability values <0.05 considered significant.

Results

The two groups did not differ in mean age (thoracic group, 61 ± 13 yr *vs.* abdominal group, 60 ± 12 yr) or weight (thoracic group, 72 ± 25 kg *vs.* abdominal group, 71 ± 12 kg). Estimated blood loss was 0.34 ± 0.32 L for thoracic patients and 0.43 ± 0.41 L for abdominal patients (NS). Abdominal group patients received more fluid (sum of crystalloid, colloid, and blood) at 3.4 ± 1.9 L compared with the thoracic group 1.1 ± 0.5 L, $P = 0.0007$). Preoperative (70 ± 3% *vs.* 72 ± 7%) and postoperative (69 ± 5% *vs.* 72 ± 6%) left ventricular ejection fraction did not differ between the thoracic and abdominal groups, respectively.

Tables 1 and 2 show the perioperative changes in time and frequency domain indices of HRV, respectively. There was a significant postoperative decrease in some time (RR, SDSD, rMSSD, pNN50) and frequency (TF, LF, HF) indices of HRV from baseline values in both groups of patients (tables 1 and 2, fig. 1). SDANN increased modestly early after surgery, but SDNN values did not change from baseline in either group (table 1). The LF:HF ratio did not change significantly from baseline values (table 2). Figure 1 shows the mean hourly spectral components of HRV over time. The two groups did not differ in any measured HRV parameter during the entire study. The corresponding hour comparison of frequency domain parameters showed decreases in TF, LF, and HF after surgery (table 3), and the LF:HF ratio did not change significantly from baseline values. The magnitude of these changes was similar to that seen when the 24-h recordings were compared with the 1-h study at baseline (table 2). Perioperative elevations in heart rate were inversely correlated to decreases in most parameters of HRV throughout the postoperative period ($P < 0.001$ by repeated-measures analysis of variance). The decrements in TF, LF, and HF were positively correlated with those of B_{max} and isoproterenol-stimulated cAMP throughout the first postoperative week ($P < 0.05$ by repeated-measures analysis of variance; fig. 2).

Table 4 shows pre- and postoperative cAMP production and βAR characteristics. Samples inadequate for receptor or cAMP analysis and patients' leaving the hospital account for fewer observations at postoperative times. There was a significant and persistent decrease in B_{max} after operation from baseline values in both groups of patients (table 4, fig. 2). Similarly, there was a significant and persistent decrease in basal and isoproterenol-stimulated cAMP activity in both groups of patients (table 4). PGE_1-stimulated cAMP activity in thoracic group patients was decreased on POD2 and POD5 and POD6 from baseline and decreased in the abdominal group patients on POD1 and POD2 (table 4). The

Table 1. Time Domain Parameters of HRV

	Preop	Op-Day	POD 1	POD 2	POD 5/6	*P* Value*
RR (ms)						
Thoracic	777 ± 136	642 ± 121	638 ± 114	628 ± 91	569 ± 80	0.0001
Abdominal	805 ± 152	682 ± 130	670 ± 140	661 ± 134	686 ± 141	
SDNN (ms)						
Thoracic	59 ± 35	50 ± 19	56 ± 18	58 ± 29	48 ± 41	0.3
Abdominal	59 ± 20	61 ± 30	50 ± 16	60 ± 23	58 ± 23	
SDANN (ms)						
Thoracic	38 ± 23	45 ± 19	50 ± 19	53 ± 33	19 ± 17	0.03
Abdominal	35 ± 13	54 ± 32	43 ± 13	53 ± 19	47 ± 19	
SDSD (ms)						
Thoracic	40 ± 26	23 ± 10	23 ± 11	19 ± 6	41 ± 48	0.002
Abdominal	45 ± 20	27 ± 13	24 ± 9	23 ± 10	29 ± 10	
rMSSD (ms)						
Thoracic	22 ± 16	14 ± 4	18 ± 11	16 ± 9	27 ± 21	0.002
Abdominal	23 ± 12	15 ± 9	14 ± 5	14 ± 6	17 ± 11	
pNN50 (%)						
Thoracic	5.5 ± 10.6	1.4 ± 2.2	3.5 ± 6.7	2.6 ± 4.9	9.7 ± 14.5	0.002
Abdominal	6.2 ± 11.1	1.5 ± 2.7	1.2 ± 1.5	1.1 ± 1.5	3.3 ± 7.4	

Preop = preoperative; Op-Day = day of operation; POD = postoperative day. Postoperative values are the mean ± SD of the hourly averages for that day. Definitions of HRV abbreviations can be found in the Appendix.

* Preoperative *versus* all postoperative time points (ANOVA). There were no differences between groups over time.

percentage decrease from preoperative values in B_{max} and isoproterenol-stimulated cAMP activity was similar in the two groups at all postoperative times. K_D varied considerably both within and between patients but showed no consistent postoperative change (table 4).

In the thoracic group there were no significant postoperative changes in the lymphocyte subset distribution, whereas in the abdominal group the percentage of B lymphocytes was minimally increased after operation (table 5). Although it was not feasible to determine receptor properties in each subpopulation of cell types given the volume of blood obtained from these patients, calculations of expected B_{max} and cAMP production based on literature values[23] for average βAR number and isoproterenol- and PGE_1-stimulated cAMP formation in individual subpopulations of lymphocytes predict

Table 2. Frequency Domain Parameters of HRV

	Preop	Op-Day	POD 1	POD 2	POD 5/6	*P* Value*
TF (ms^2)						
Thoracic	929 ± 1,366	254 ± 192	218 ± 162	163 ± 109	235 ± 333	0.0001
Abdominal	1,168 ± 1,068	348 ± 359	267 ± 204	244 ± 208	289 ± 223	
LF (ms^2)						
Thoracic	358 ± 555	89 ± 100	63 ± 50	45 ± 30	60 ± 86	0.0001
Abdominal	529 ± 605	95 ± 93	90 ± 74	74 ± 67	83 ± 54	
HF (ms^2)						
Thoracic	136 ± 310	22 ± 8	34 ± 51	29 ± 36	58 ± 114	0.002
Abdominal	110 ± 113	31 ± 41	30 ± 36	28 ± 28	46 ± 60	
LF/HF ratio						
Thoracic	4.4 ± 2.8	4.2 ± 4.6	3.5 ± 3.8	3.0 ± 3.1	2.5 ± 2.5	0.65
Abdominal	5.1 ± 3.1	4.5 ± 4.6	4.6 ± 4.5	2.9 ± 1.2	3.2 ± 1.9	

Preop = preoperative; Op-Day = day of operation; POD = postoperative day. Postoperative values are the mean ± SD of the hourly averages for that day. Definitions of HRV abbreviations can be found in the Appendix.

* Preoperative *versus* all postoperative time points (ANOVA). There were no differences between groups over time.

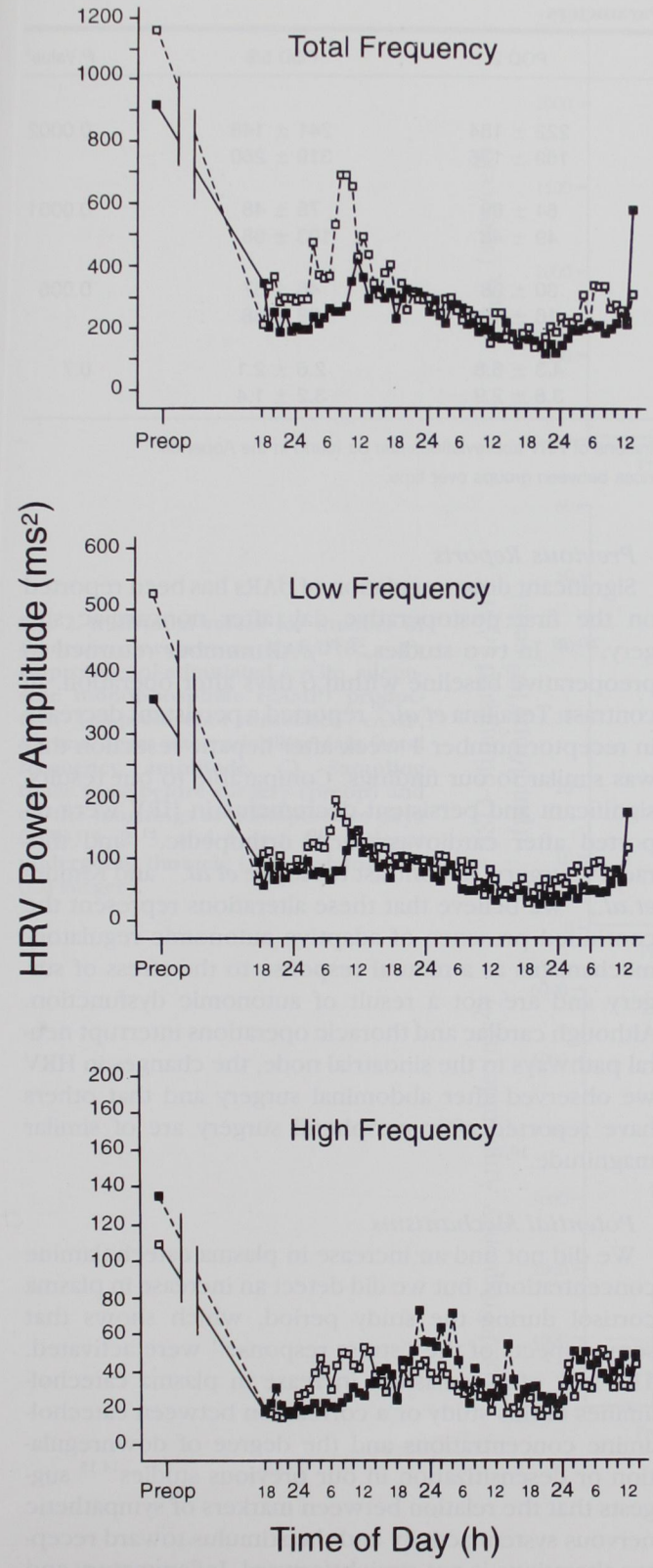

Fig. 1. Circadian changes in frequency domain parameters of HRV after abdominal (□) and thoracic (■) surgery. Data are hourly mean values obtained before surgery (preop) and starting at 6:00 P.M. on the day of surgery for approximately 72 h after operation.

that no meaningful postoperative change in receptor density or cAMP production would occur as a result of this minor subpopulation alteration.

Table 6 shows plasma epinephrine, norepinephrine, and cortisol concentrations at the times studied. Plasma epinephrine and norepinephrine concentrations did not change significantly from baseline values in either group of patients. Plasma cortisol concentrations in both groups were significantly greater than baseline at postoperative sampling periods. The concentrations of plasma epinephrine, norepinephrine, and cortisol did not differ between the two groups at any time.

Within the thoracic group there were no significant differences in βAR function, plasma catecholamines, or HRV parameters between the subset of patients who received intravenous patient-controlled analgesia for pain relief (n = 6) compared with those who received a thoracic epidural infusion (n = 8).

Discussion

In response to elective major noncardiac surgery, significant and persistent decrements in most parameters of HRV were seen during the first postoperative week. These alterations correlated with βAR/adenylyl cyclase downregulation and desensitization in the absence of significant change in plasma catecholamine concentrations or cardiac function as determined by echocardiography. Plasma cortisol concentrations were increased to a level consistent with mild postoperative stress in both groups. Exogenous glucocorticoids have been shown to reverse agonist-induced downregulation of lymphocyte βAR function,[24] but the role, if any, of cortisol in βAR regulation after surgery is uncertain. In this and other studies,[14,15] we examined the percentage distribution of lymphocyte subsets (B cells, subpopulations of T cells, natural killer cells, and so on) at each time point, because it was shown that these subsets differ in βAR properties, and alterations in subpopulation ratios could be misinterpreted as alterations in receptor function.[23] The lack of significant change in subpopulation ratios in any of our studies suggests that

Table 3. Corresponding Hour Comparison of Frequency Domain Parameters

	Preop	POD 1	POD 2	POD 5/6	*P* Value*
TF (ms^2)					
Thoracic	929 ± 1,366	367 ± 372	222 ± 184	241 ± 148	0.0002
Abdominal	1,168 ± 1,068	309 ± 267	169 ± 136	319 ± 260	
LF (ms^2)					
Thoracic	358 ± 555	108 ± 117	64 ± 69	75 ± 48	0.0001
Abdominal	529 ± 605	107 ± 107	49 ± 48	103 ± 88	
HF (ms^2)					
Thoracic	136 ± 310	29 ± 20	30 ± 38	46 ± 57	0.006
Abdominal	110 ± 113	35 ± 43	16 ± 12	38 ± 38	
LF/HF ratio					
Thoracic	4.4 ± 2.8	3.6 ± 3.3	4.3 ± 6.8	2.6 ± 2.1	0.7
Abdominal	5.1 ± 3.1	5.7 ± 9.3	3.8 ± 2.9	3.2 ± 1.4	

Preop = preoperative; Op-Day = day of operation; POD = postoperative day. Definitions of HRV abbreviations can be found in the Appendix.

* Preoperative *versus* all postoperative time points (ANOVA). There were no differences between groups over time.

the receptor alterations are indeed due to changes in individual lymphocyte function.

With the exception of SDNN and SDANN, all other time (RR, SDSD, rMSSD, pNN50) and frequency (TF, LF, HF) domain parameters of HRV decreased significantly in response to surgical stress and are consistent with sympathetic hyperactivity. The lack of change in SDNN in our patients may be explained by the fact that baseline (1 h) SDNN values were already reduced compared with normal (24 h) values (>100 ms),[6,7,9] most likely because of patient preoperative anxiety. Despite a small statistical increase in SDANN after surgery, the values were indicative of very low HRV, as were those of SDSD. SDANN is more likely to react to changes caused by posture, activity, and day-night differences, whereas SDSD is sensitive to high-frequency components of HRV and less so to posture, activity, or circadian rhythms, all of which can be affected by surgery. For these reasons, SDSD may be a more useful indicator of HRV in the postoperative period. The LF:HF values seen before and after surgery in our patients are comparable to those described in persons not undergoing surgery.[6,8] It is unlikely that the postoperative LF:HF ratio represents the true physiologic sympathovagal relation in patients having surgery with severely restricted overall variance of HRV.[25] Perioperative changes in heart rate were inversely correlated with those seen in most time or frequency domain parameters of HRV. Although the intrinsic heart rate changes with age, physical activity, right atrial wall stretch, and other physiological modifiers, we agree with other investigators[26] who suggest that heart rate may be used as an index of sympathovagal balance.

Previous Reports

Significant downregulation of βARs has been reported on the first postoperative day after noncardiac surgery.[27-29] In two studies,[27,29] βAR number returned to preoperative baseline within 6 days after operation. In contrast, Terajima *et al.*[30] reported a persistent decrease in receptor number 1 week after hepatic resection that was similar to our findings. Comparable to our results, significant and persistent decrements in HRV were reported after cardiovascular,[10] orthopedic,[11] and thoracic[12] surgery. In contrast to Hogue *et al.*[10] and Kimura *et al.*,[12] we believe that these alterations represent the continued presence of adaptive autonomic regulatory mechanisms as a natural response to the stress of surgery and are not a result of autonomic dysfunction. Although cardiac and thoracic operations interrupt neural pathways to the sinoatrial node, the changes in HRV we observed after abdominal surgery and that others have reported after peripheral surgery are of similar magnitude.[10,11]

Potential Mechanisms

We did not find an increase in plasma catecholamine concentrations, but we did detect an increase in plasma cortisol during the study period, which shows that some aspects of the "stress response" were activated. The lack of a consistent increase in plasma catecholamines in this study or a correlation between catecholamine concentrations and the degree of downregulation or desensitization in our previous studies[14,15] suggests that the relation between markers of sympathetic nervous system activity and the stimulus toward receptor alterations is not straightforward. Inflammatory and

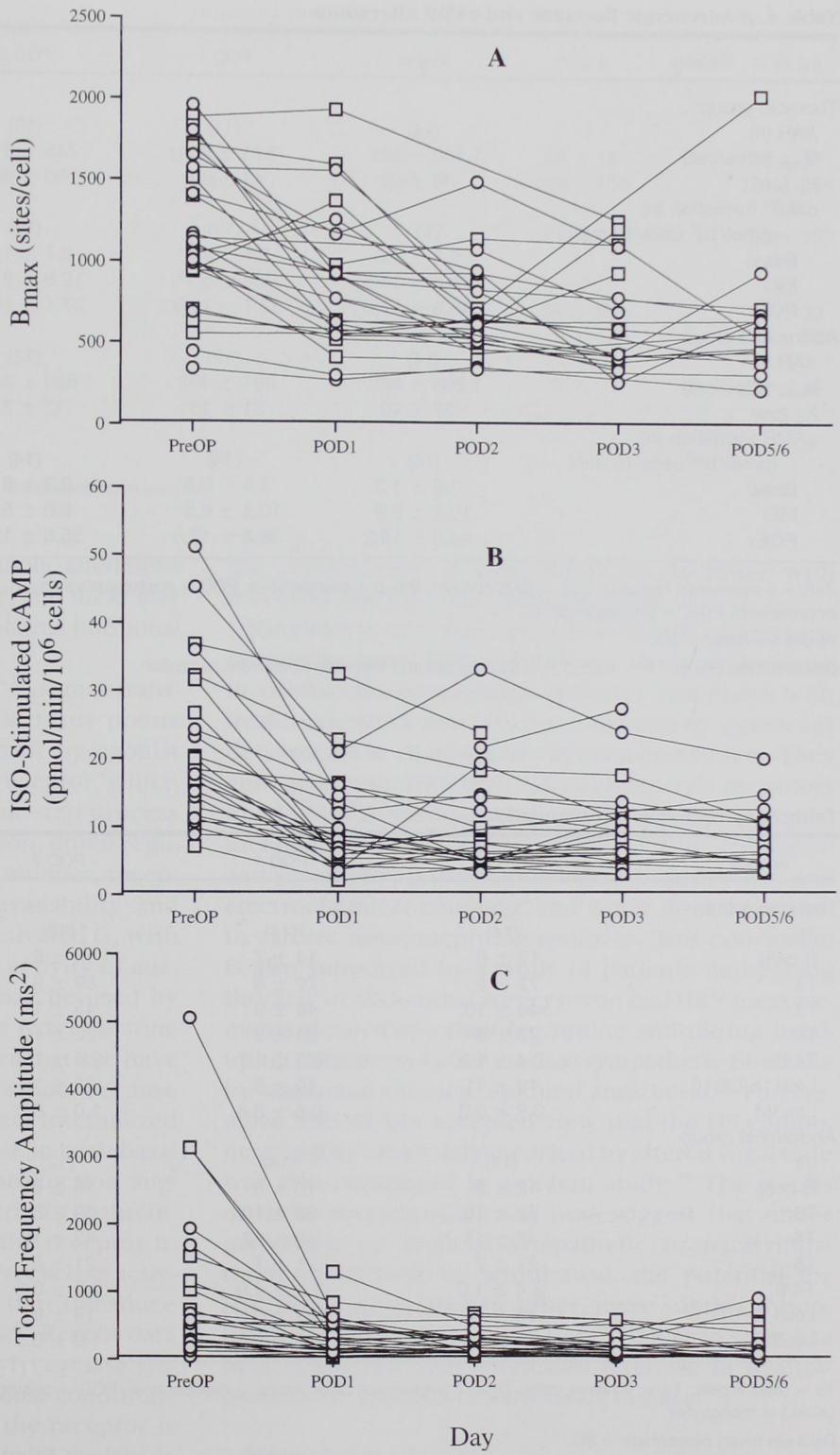

Fig. 2. Individual values for number of β-adrenergic receptors per cell (B_{max}, *A*), isoproterenol-stimulated cyclic adenosine monophosphate (cAMP) production (isoproterenol-stimulated cAMP, *B*), and heart rate variability data (total frequency amplitude, *C*). Sampling times were before surgery (preop) and on the mornings of postoperative days (POD) 1, 2, 3, and 5 or 6 for patients undergoing thoracic (○) or abdominal (□) surgery.

Table 4. β-Adrenergic Receptor and cAMP Alterations

Variable	Preop	POD 1	POD 2	POD 3	POD 5/6
Thoracic group					
βAR (n)	(14)	(11)	(13)	(8)	(9)
B_{max} (sites/cell)	1,169 ± 481	813 ± 406†	745 ± 318*	569 ± 239*	561 ± 269*
K_D (pM)	49 ± 58	43 ± 68	50 ± 64	59 ± 54	51 ± 52
cAMP formation (n)					
(pmol/10^6 cells/10 min)	(11)	(11)	(11)	(7)	(9)
Basal	5.3 ± 2.0	3.1 ± 1.2*	2.7 ± 1.1*	2.6 ± 0.6*	2.8 ± 1.2*
ISO	23.4 ± 14.5	10.4 ± 5.1*	12.8 ± 9.2*	11.8 ± 9.2	9.5 ± 5.6*
PGE_1	46.6 ± 17.0	32.7 ± 13.0	27.1 ± 10.8*	31.0 ± 10.5	35.4 ± 10.8*
Abdominal group					
βAR (n)	(14)	(12)	(12)	(9)	(8)
B_{max} (sites/cell)	1,206 ± 462	897 ± 492	629 ± 208*	695 ± 511	802 ± 377*
K_D (pM)	27 ± 40	21 ± 16	12 ± 7	9 ± 4	16 ± 9
cAMP formation (n)					
(pmol/10^6 cells/10 min)	(14)	(14)	(14)	(10)	(9)
Basal	3.8 ± 1.3	2.5 ± 0.8*	2.3 ± 0.6*	2.5 ± 07*	1.9 ± 0.4*
ISO	19.9 ± 8.9	10.2 ± 8.5*	8.5 ± 5.8*	8.6 ± 4.2*	6.8 ± 2.7*
PGE_1	48.0 ± 16.2	38.8 ± 12.5	35.6 ± 12.7*	41.9 ± 22.1	34.5 ± 11.1

βAR = β-adrenergic receptor; B_{max} = βAR number; Preop = preoperative; POD = postoperative day; K_D = dissociation constant; cAMP = cyclic AMP; ISO = isoproterenol; PGE_1 = prostaglandin E_1.

Values are mean ± SD.

Different from Preop: * $P < 0.05$, † $P = 0.06$ by paired t test with Bonferroni correction.

Table 5. Lymphocyte Subpopulations

Antigen	Preop	POD 1	POD 2	POD 3	POD 5/6
Thoracic group					
(n)	(14)	(14)	(13)	(10)	(12)
B cells	12 ± 6	14 ± 7	15 ± 8	14 ± 8	13 ± 7
T3	72 ± 5	70 ± 6	69 ± 9	70 ± 8	73 ± 5
T4	49 ± 10	46 ± 9	45 ± 11	47 ± 9	52 ± 8
T8	23 ± 8	23 ± 9	22 ± 10	20 ± 9	21 ± 8
T4/8	2.4 ± 1.2	2.5 ± 1.7	2.5 ± 1.4	2.8 ± 1.5	3.0 ± 1.6
Leu11b/CD16	19 ± 11	19 ± 9	22 ± 12	17 ± 9	17 ± 9
Leu3M	0.9 ± 0.3	0.8 ± 0.4	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Abdominal group					
(n)	(14)	(14)	(12)	(10)	(9)
B cells	12 ± 8	16 ± 8	16 ± 8*	13 ± 5	11 ± 5
T3	72 ± 10	68 ± 10	69 ± 8	71 ± 12	72 ± 9
T4	47 ± 13	42 ± 12	43 ± 10	48 ± 12	50 ± 10
T8	21 ± 7	21 ± 7	21 ± 6	19 ± 7	20 ± 7
T4/8	2.4 ± 1.0	2.1 ± 1.0	2.2 ± 0.8	3.0 ± 1.3	2.6 ± 1.2
Leu11b/CD16	18 ± 12	21 ± 9	19 ± 9	20 ± 10	21 ± 10
Leu3M	1.1 ± 0.5	0.9 ± 0.3	1.0 ± 0.5	0.8 ± 0.5	0.8 ± 0.5

T3 = total T cells; T4 = T helper cells; T8 = T suppressor cells; Preop = preoperative; POD = postoperative day; Leu11b/CD16 = natural killer (NK) cells; LeuM3 = monocytes.

Data are mean percentage ± SD.

* Different from Preop, $P < 0.05$ by paired t test with Bonferroni correction.

Table 6. Humoral Alterations

	Preop	POD 1	POD 2	POD 3	POD 5/6
Thoracic group					
N	9	9	8	7	7
Epinephrine (pg/ml)	41 ± 18	77 ± 117	27 ± 17	26 ± 15	36 ± 37
Norepinephrine (pg/ml)	391 ± 235	342 ± 368	265 ± 143	288 ± 158	378 ± 354
N	14	14	13	11	11
Cortisol (μg/dl)	14 ± 6	24 ± 11*	22 ± 8	21 ± 10	25 ± 10*
Abdominal group					
N	10	10	10	7	5
Epinephrine (pg/ml)	40 ± 29	100 ± 64	114 ± 149	56 ± 36	76 ± 50
Norepinephrine (pg/ml)	214 ± 92	460 ± 382	536 ± 413	316 ± 145	567 ± 354
N	14	14	14	9	9
Cortisol (μg/dl)	16 ± 5	21 ± 5*	22 ± 6	22 ± 11	23 ± 10

Preop = preoperative; POD = postoperative day.

Values are mean ± SD.

* Different from Preop ($P \leq 0.05$) by paired t test with Bonferroni correction.

immunologic responses and other metabolic alterations are also known to occur perioperatively, and these may or may not be reflected by traditional plasma hormonal and neurotransmitter measurements.[31]

As we have discussed previously,[14,15] signal transduction *via* the βAR may be altered at many points in the cascade. Occupation of the βAR by agonist leads to rapid phosphorylation of the receptor, which appears to be the first step in a multiple-step process that can lead ultimately to desensitization, down-regulation,[32] or both. Changes in receptor number, receptor internalization (sequestration), availability and coupling ability of G_s, interaction of activated G_S with adenylyl cyclase, and the amount and activity of adenylyl cyclase present in the cell all may be used by the cell to regulate responsiveness to external stimuli.[33] The decrease in receptor number that we have detected cannot be due to internalization because iodopindolol is lipophilic and will detect internalized receptors. The similar 40–50% decrease in both basal and isoproterenol-stimulated cAMP production suggests that the major "defect" in the βAR-G-protein-adenylyl cyclase pathway is distal to the receptor itself. Alterations in G-protein or adenylyl cyclase activity have wide-ranging effects, because they transduce signals for a wide variety of receptors.[34] Recent data on the stoichiometry of the βAR-adenylyl cyclase system in myocytes suggests that under most conditions the rate-limiting component distal to the receptor is the adenylyl cyclase enzyme.[35] If adenylyl cyclase is altered in the postoperative period, the phenomenon of decreased cellular responsiveness to agonists in the postoperative period would be operative with receptor systems other than the βAR.

Kingwell *et al.*[36] showed that although patients experiencing heart failure did have a threefold increase in cardiac norepinephrine spillover compared with healthy persons, this was accompanied by significant decrements in all frequency domain parameters. They concluded that HRV at 0.1 Hz (LF) depends on factors in addition to cardiac sympathetic nerve firing rates, including multiple neural reflexes, βAR regulation and sensitivity, postsynaptic signal transduction, and electrochemical coupling, and is not directly related to cardiac norepinephrine spillover. This conclusion is also supported by a study of patients undergoing thoracic or abdominal surgery who had HRV measurements done while they lay supine and during head-up tilt, before and after cardiac sympathetic blockade by segmental thoracic epidural anesthesia.[37] Furthermore, the widely accepted view that the HF component of HRV was solely mediated by altered vagal tone was also challenged in a recent study.[38] The results of these investigations and ours suggest that under pathologic or artificial sympathetic or parasympathetic stimulation or withdrawal, the potential for HRV to be modulated by other, more subtle, physiologic control mechanisms is lost,[39] similar to what has been described after strenuous exercise in healthy persons or in patients with heart disease.[25,39,40]

Limitations

The small sample size used in this study to determine trends in βARs and HRV in response to major noncar-

diac surgery was not meant to establish "normal" values for these indices in the perioperative period. Our results may not apply to patients with illnesses that might affect autonomic state and regulation, such as congestive heart failure, severe diabetes mellitus, and so on. Although there are significant limitations to the lymphocyte model, which we have previously described,[14,15] frequently these βARs are used as a model for cardiovascular and other βARs because of the ease of obtaining lymphocytes repeatedly. The significant alterations we have found show that cell-surface receptor regulatory mechanisms are activated by events during the perioperative period. Linear methods of HRV analysis (Fast Fourier) may be influenced by several important conditions, including age, posture, respiratory rate, circadian rhythms, disease states, and medications. Posture and respiratory rate could not be controlled in either abdominal or thoracic patients, but other variables were constant. All of our patients were treated with opioids after operation, which may have affected HRV by modulating pain level and respiratory rate. We did not observe any significant differences in βAR and HRV data between the subset of patients receiving epidural opioids and those who did not after thoracic surgery. As a general rule, sequential HRV studies should be performed during a similar time period.[9] Because patients are no longer admitted the night before elective surgery, we did not obtain 24-h baseline HRV recordings, and thus 1-h baseline values may not be truly representative. However, our preoperative (1-h) baseline frequency domain parameters were compared with data obtained from both 1-h and 24-h postoperative recordings. To our knowledge, the finding that the results from a separate analysis using the corresponding hour were nearly identical to those obtained when 24-h recordings were used is novel. We could not make similar comparisons in time-domain parameters because of the limitations of the commercial software used for HRV analysis.

Clinical Implications and Conclusions

In patients without known organic heart disease, significant and persistent decrements in time and frequency domain parameters of HRV correlated with alterations in lymphocyte βAR system function in response to abdominal or thoracic surgery during the first postoperative week. These changes likely represent appropriate physiologic defense mechanisms to potent noxious stimuli, to prevent or attenuate exaggerated end-organ effects, and functionally may be similar to what clinicians try to achieve pharmacologically with perioperative β-blockade[41] and α_2-agonist administration.[42] The fact that these alterations in βAR function and HRV persist during the first postoperative week adds biochemical and physiologic evidence to a growing clinical perception that the postoperative period may be more critical than the intraoperative period in determining ultimate clinical outcomes. In contrast to postmyocardial infarction patients, in whom low HRV predicts decreased survival,[6-8] restricted variance in most patients undergoing surgery is a common occurrence.[10-12] The cardioprotective effects of β-adrenergic blocking drugs on perioperative cardiac events[41] may be reflected in their abilities to increase HRV by slowing the sinus rate and increasing vagal tone.[43]

Appendix: Definitions of Heart Rate Variability Parameters

Time Domain Normal 24-h Values[6]

RR: R to R interval, 817 ± 103 ms

SDNN: Standard deviation of all RR intervals, 141 ± 39 ms

SDANN: Standard deviation of 5-min mean RR intervals, 127 ± 35 ms

SDSD: Mean of all 5-min SDs of RR intervals, 54 ± 15 ms

rMSSD: Root-mean square of difference of successive RR intervals, 27 ± 12 ms

pNN50: Proportion of adjacent RR intervals more than 50 ms different, 9 ± 7%

Frequency Domain

TF: total frequency amplitude (0.01 - 1.0 Hz), 21,222 ± 11,663 ms^2 (<0.40 Hz)[6]

LF: low frequency (0.04 - 0.15 Hz), 791 ± 563 ms^2

HF: high frequency (0.15 - 0.40 Hz), 229 ± 282 ms^2

The SDANN index shows how much the heart rate during each 5-min period differs from the overall day-long mean heart rate.[9] In contrast to SDANN, the SDSD index shows how much variation occurs within 5-min periods and tends to ignore variations that develop over longer periods. The rMSSD and pNN50 both reflect high-frequency components of HRV that are heavily influenced by vagal tone.[9]

High-frequency power is thought to represent pure parasympathetic signal reflecting respiratory sinus arrhythmia. Low-frequency power reflects both parasympathetic and sympathetic modulation of heart rate and is strongly influenced by baroreflex activity. The LF:HF ratio has been proposed as an index of sympathovagal balance.[8,9,26] The physiologic processes responsible for modulating very low frequency power (<0.15 Hz) are not known, although speculation includes temperature regulation and fluctuations in the activity of the renin-angiotensin system.

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