A number of clinical case reports and studies on animal models have provided evidence that psychological stressors of diverse types may lower the cardiac threshold for severe ventricular events, especially when these stressors act on an altered cardiac substrate resulting from myocardial consequences of coronary artery disease, left ventricular hypertrophy, and myopathic ventricles (18, 26, 38). The effects of psychological events on cardiac electrical activity are mediated by the interaction of the two branches of the autonomic nervous system. An increased sympathetic activity is known (40) to lower the vulnerable-period threshold for ventricular fibrillation, whereas vagal prevalence is associated with a reduced risk of arrhythmias. Because ventricular premature beats are risk markers for malignant ventricular arrhythmias and sudden cardiac death (20), a relevant question is to what extent their frequency and severity are affected by different psychological stressors.

Emotional stressors of different natures can induce different shifts of autonomic balance, toward either a sympathetic or a parasympathetic prevalence, that also depend on the level of induced physical activity. As originally pointed out by Obrist (24), with a passive behavioral coping strategy such as the one produced by classic aversive conditioning, the heart is more under vagal control, whereas with active coping such as shock avoidance, the heart is under greater sympathetic control.

Lown and Verrier and co-workers (16, 19, 36, 38) developed behavioral models of aversive conditioning, anger, and fear in dogs, and they showed the efficacy of these psychological stress paradigms in decreasing the threshold for severe ventricular events during acute myocardial ischemia and infarction.

Other studies (8, 12) investigated the occurrence of arrhythmias associated with hypothalamically induced emotional behaviors under light anesthesia in normal cats. Ventricular arrhythmias were frequently observed in emotional behaviors such as restlessness and threat but rarely in sniffing or biting behavior.

Only a few studies examined in detail cardiac electrophysiologic responses to acute social stressors in normal animals, i.e., with no present or previously induced cardiovascular pathology. For this purpose, the rat may represent a valid animal model; social aversive events such as defeat, psychosocial stimulation, and maternal aggression can be reliably reproduced in the laboratory, and they have been proved to induce significant acute neuroendocrine and cardiovascular responses (2, 13, 22, 28).

Among these models of social stress, defeat was shown to be the most effective in producing acute elevations of blood pressure and plasma catecholamine and corticosterone levels in laboratory rats (13). Especially when applied to a wild-type strain of rats, this stress paradigm was shown to produce a strong activation of both the sympathetic adrenomedullary system and the pituitary-adrenocortical axis (27).

The aim of the present study was to provide a detailed description of the acute effects of social defeat on heart rate, heart rate variability, and arrhythmia...
occurrence in male members of a wild-type strain of rats. An additional objective was to compare these effects with those obtained with a nonsocial stress paradigm such as restraint, a widely used emotional stress model with proven impact on the sympathetic adrenomedullary system and the pituitary-adrenocortical axis (25). Our assumption was that the two stressors could differ in terms of autonomic control of cardiac electrical activity and therefore represent psychological factors with different arrhythmogenic potential.

Cardiac electrical activity was obtained via miniature telemetry transmitters that allow reliable measurements during free agonistic interactions between the animals (3, 30). Heart rate variability, which provides information on neural (autonomic) control of the heart, was quantified with the use of the following time-domain parameters: standard deviation (SD) of average R-R interval (RR), coefficient of variance (SD/RR), and root mean square of successive RR differences (r-MSSD) (9, 33, 35).

These parameters have been shown to correlate well with frequency-domain measurements (33). In particular, the SD and the SD/RR, which provide the same information given by the total power of the spectrum of RR variability, are both measures of the total variance in the heart rate signal. Therefore, they give an overall estimation of the balance between the sympathetic and parasympathetic activities on the heart. The r-MSSD, which correlates highly with the high-frequency power of the spectrum, more specifically quantifies the vagal influence on heart rate variability (33).

An indirect estimation of the sympathetic input to the heart during social stress and restraint was also obtained via determination of plasma catecholamine concentrations.

METHODS

All procedures in this study were approved by the Committee on Animal Bioethics of the University of Groningen, The Netherlands.

Animals and Housing

We used 56 male wild-type rats (Rattus norvegicus). They were housed in unisexual groups of five individuals, from weaning until the onset of experiments (6 mo of age), in clear Plexiglas cages measuring 25 × 20 × 20 cm. Ten additional males were used as resident dominants in the social stress test ("resident-intruder test", see Social Stress and Restraint Procedures for details). Each resident was permanently housed with a female in a Plexiglas cage (25 × 25 × 30 cm) to induce and preserve high levels of aggression toward strange male conspecific intruders (14). Experimental and dominant males were kept in separate rooms with controlled temperature (22 ± 2°C) and lighting (lights on from 8:00 PM to 8:00 AM). The bedding in the cages consisted of wood shavings, and food and water were freely available.

Telemetry System

The telemetry system employed in this study consisted of a flat transmitter measuring 25 × 15 × 8 mm (TA11CTA-F40, Data Sciences International, St. Paul, MN) and a platform receiver measuring 35 × 22 × 3 cm and manufactured by the electronics department in the Biological Center of the University of Groningen (The Netherlands).

Surgeries

Transmitter implantation. In 33 males, the telemetry electrocardiogram (ECG) transmitter was chronically implanted after a surgical procedure that guarantees high-quality ECG recordings even during sustained physical activity (30). Briefly, the body of the transmitter was placed in the abdominal cavity, and the two electrodes (wire loops) were fixed to the dorsal surface of the xiphoid process and to the anterior mediastinum close to the right atrium.

Jugular vein cannulation. The remaining 23 males were provided with a Silastic heart cannula (ID 0.5 mm, OD 0.9 mm; Dow Corning, Midland, MI) through the right jugular vein, with one end reaching the entrance of the right atrium and the other end externalized on top of the skull, according to the technique originally described by Steffens (32).

Surgeries were performed under halothane anesthesia (Fluothane, Zeneca, Ridderkerk, The Netherlands). Subsequently, rats were prophylactically injected with natrium penicillin G (20 IU/kg body wt sc; Yamanouchi, Leiderdorp, The Netherlands) and individually housed in clear Plexiglas cages measuring 25 × 25 × 30 cm.

Social Stress and Restraint Procedures

Social stress (SS) and restraint (RS) challenges were performed 10 days after ECG transmitter or jugular vein cannula implantation. Animals were randomly assigned to either an SS or RS group. Each recording session consisted of a baseline and a test period lasting 15 min each. One hour before baseline, either the telemetry transmitter was switched on or a long polyethylene tube was connected to the externalized end of the catheter for blood withdrawal. During baseline, experimental animals were left undisturbed in their own home cages. Subsequently, SS rats (25 bearing the ECG transmitter and 15 bearing the jugular cannula) were individually introduced into the home cage of an aggressive resident (trained fighter) after temporary removal of the female partner (resident-intruder test), where they were vigorously attacked (1). RS rats (8 bearing the transmitter and 8 bearing the cannula) were introduced in a Plexiglas tube (ID 6 cm, length 20 cm), closed at both ends by removable partitions provided with holes (diameter 5 mm). From telemetered rats, ECGs were continuously recorded during baseline and test periods. From cannulated rats, blood samples of 0.5 ml were withdrawn at 5 and 15 min into baseline and at 1, 5, and 15 min into the test periods. To measure the recovery of basal catecholamine levels, we also took blood samples 15 and 45 min after test termination, with the animal back in its home cage. After each sample was taken, the same amount of donor blood was transfused through the catheter to avoid changes in hemodynamics. Donor blood was obtained from additional unstressed rats provided with permanent heart catheters. All SS and RS experimental sessions were performed between 10:00 AM and 1:00 PM.

ECG Data Acquisition and Processing

The pulse-modulated signal at the output of the receiver was simultaneously routed to two IBM-compatible personal computers (PC). One PC was host to a software package developed in our lab (CARDIA) for real-time acquisition and analysis of R-R intervals. R waves were converted into pulses using a threshold circuit. Pulse times were measured with <0.5 ms accuracy. R-R pulse intervals were expressed as heart rate (beats/min), displayed on line, and stored for
subsequent analysis. The second PC contained the LABPRO data-acquisition system (Data Sciences, St. Paul, MN), which was used only for monitoring, storage, and visual inspection of ECG waves. The following ECG parameters were quantified: 1) mean RR in milliseconds; 2) variability of RR measured in the time domain and expressed as SD, SD/RR (coefficient of variance) (9), and r-MSSD in milliseconds (33); and 3) number of arrhythmic events, such as ventricular (VPB) and supraventricular premature beats (SPB) and supraventricular tachycardias (VT) (4). Whereas SD and SD/RR estimate the overall heart rate variability and therefore include the contribution of both branches of the autonomic nervous system to heart rate control, r-MSSD specifically quantifies the influence on heart rate variability of the parasympathetic input to the heart (33, 35). Mean RR and RR variability measures were performed after removal of R-R intervals surrounding arrhythmias.

Catecholamine Determination

Blood samples were immediately transferred to chilled (0°C) centrifuge tubes containing EDTA and 10 mL heparin solution (500 IU/mL). Blood was centrifuged at 5°C for 10 min at 2,600 revolutions/min, and 100 µl of the supernatant were stored at −80°C. Determination of plasma catecholamine concentrations was performed by means of high-performance liquid chromatography in combination with electrochemical detection according to the technique described by Smedes and colleagues (31).

Statistical Analysis

Quantification of aggressive interactions during the resident-intruder test was limited to the number of attacks received by each intruder and the latency (in seconds) to the first biting attack.

ECGs were recorded from 25 animals during defeat and 8 animals during restraint. Blood samples were withdrawn from an additional 15 males during defeat and 8 males during restraint. R-R interval measurements during social stress and restraint (mean RR, SD, SD/RR, and r-MSSD; see ECG Data Acquisition and Processing) were calculated as means of 15- or 1-min periods. For all these parameters, means of 15-min periods were compared via a one-way analysis of variance (ANOVA), whereas comparison of means of 1-min periods between defeat and restraint was performed with the use of a two-way ANOVA, with stress treatment as a between-subject factor (2 levels) and time as a repeated-measures within-subject factor (15 levels). Arrhythmia occurrence was expressed as either the number of events per each 15-min recording period (baseline and test periods) or the number of events per minute of the test period. For each cannulated animal, the two baseline measurements of catecholamines were averaged, and only mean values were used for statistical analysis. The response patterns of each hormone were first evaluated with the use of a two-way ANOVA, with stress treatment as a between-subject factor (2 levels) and sampling time as a repeated-measures within-subject factor (6 levels). Plasma catecholamine responses were also quantified by computing the area under the response time curve (AUC) above the baseline. The AUC values were statistically analyzed by means of a one-way ANOVA. Further post hoc analyses on electrocardiographic and humoral data were performed by means of Scheffé’s test. Values for electrocardiographic parameters together with plasma levels and AUC values for catecholamines and behavioral patterns were expressed as means ± SE.

RESULTS

Aggressive Interactions During Defeat

During the 15-min resident-intruder test, intruders received on average 6 ± 1 attacks. The mean latency time to the first biting attack was 60 ± 14 s. The aggressive behavior toward the intruders was exhibited by the dominants throughout the test duration.

Electrocardiographic Responses

Mean RR and RR variability. Mean RR values were significantly decreased during both social defeat and restraint compared with baseline (P < 0.01), but the heart rate acceleration observed during defeat was significantly more pronounced (Table 1; P < 0.02). RR variability, measured as SD of the mean of 15-min stress periods, was significantly decreased during defeat but increased during restraint compared with baseline (Table 1; P < 0.02). RR variability expressed as SD corrected by the heart rate (coefficient of variance expressed as SD/RR) was unchanged during defeat compared with baseline but markedly increased during restraint (Table 1; P < 0.01). With the use of r-MSSD, which specifically quantifies the short-term components of RR variability (parasympathetic input to the heart), significantly higher values were found during restraint compared with baseline and defeat (Table 1; P < 0.05). Figure 1 reports the minute-by-minute time evolution of mean RR and high-frequency RR variability (r-MSSD) during defeat and restraint. Mean RR and r-MSSD were significantly lower during defeat, starting from the fourth and third minutes of the tests, respectively (P < 0.05).

Arrhythmias. Figure 2 reports examples of the arrhythmic events recorded during the experimental sessions. The occurrence of VPB and SPB was significantly higher during defeat compared with baseline conditions and RS (VPB: SS = 7.4 ± 1.3 vs. RS = 0.8 ± 0.3 and baseline = 0.1 ± 0.1, P < 0.01; SPB: SS = 4 ± 1.4 vs. RS = 0.9 ± 0.2 and baseline = 0.3 ± 0.3, P < 0.05). Events of VT and SVT were very rare (2 episodes of each), all occurring during defeat (Fig. 2).

Table 1. Changes in heart rate and heart rate variability during social stress and restraint

<table>
<thead>
<tr>
<th></th>
<th>RR, ms</th>
<th>SD, ms</th>
<th>SD/RR</th>
<th>r-MSSD, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>Baseline</td>
<td>167 ± 2.4</td>
<td>10.9 ± 0.6</td>
<td>0.066 ± 0.003</td>
</tr>
<tr>
<td>Test</td>
<td>122 ± 1.7*</td>
<td>8.1 ± 0.67</td>
<td>0.065 ± 0.004</td>
<td>2.49 ± 0.13</td>
</tr>
<tr>
<td>RS</td>
<td>Baseline</td>
<td>170 ± 3.1</td>
<td>9.8 ± 1.33</td>
<td>0.068 ± 0.003</td>
</tr>
<tr>
<td>Test</td>
<td>136 ± 4.5*</td>
<td>15.8 ± 1.4*</td>
<td>0.114 ± 0.011*</td>
<td>3.30 ± 0.45*</td>
</tr>
</tbody>
</table>

Values are means ± SE. RR, average R-R interval; SD, standard deviation; SD/RR, coefficient of variance; r-MSSD, root mean square of successive differences; SS, social stress (n = 25 rats); RS, restraint (n = 8 rats). *P < 0.01, significant difference between test and baseline values, one-way ANOVA; †P < 0.05, significant difference between SS and RS test values, one-way ANOVA; ‡P < 0.05, significant difference between test and baseline and between SS and RS test values.
Among all VPB observed during SS (n = 185, the most common type of arrhythmia documented in this study), ~70% occurred in the 1-min windows of time following attacks. Expressed as the number of events per minute per animal, this frequency of events was significantly higher than that during the 1-min windows of time preceding attacks (Table 2; P < 0.01). These “critical” 1-min periods were also characterized by significantly lower values of mean RR and SD than those during the 1-min periods preceding attacks (Table 2; RR, P < 0.01; SD, P < 0.05), whereas r-MSSD values were similar before and after attacks.

**Plasma Catecholamine Levels**

Figure 3 shows the mean time course of plasma concentrations of catecholamines during SS and RS. Both stressors induced a significant increase in catecholamines over baseline. During SS, norepinephrine (NE) and epinephrine (Epi) were significantly elevated at t = 1, 5, 15, and 30 min (P < 0.05) and maximum peak values were found at 5 min for both catecholamines (NE: 1,284 ± 548 pg/ml; Epi: 1,087 ± 251 pg/ml). In RS animals, catecholamine increments were also significant at t = 1, 5, 15, and 30 min (P < 0.05, except at t = 30 min for Epi, which was back to baseline). However, unlike SS, peak values during RS were observed at the beginning of the test (t = 1 min; NE: 1,159 ± 122 pg/ml, Epi: 473 ± 82 pg/ml). Significant differences between corresponding time points in the two stress conditions were found at t = 5, 15, and 30 min for Epi (P < 0.01) and t = 1, 5, and 15 min for NE (P < 0.05). A quantitative overall comparison between the two stress situations was obtained by using AUC values of the two hormones. SS induced a significantly higher elevation of Epi (SS = 32,823 ± 9,535 vs. RS = 6,467 ± 624 pg·ml⁻¹·min⁻¹, P < 0.05), whereas the NE response, although also tendentially higher, was not statistically different (SS = 41,344 ± 5,945 vs. RS = 28,496 ± 4,914 pg·ml⁻¹·min⁻¹, P = 0.07).

**DISCUSSION**

In this study on healthy wild-type rats, acute electrocardiographic and plasma catecholaminergic responses to social defeat are described and compared with those observed in the nonsocial aversive context of restraint.

Defeat produced a stronger heart rate increase and a much higher incidence of arrhythmic events. Time-
domain heart rate variability measurements and plasma catecholamine concentrations suggest that 1) during defeat, a much higher activation of the sympathetic component of the autonomic nervous system takes place than during restraint and 2) this sympathetic activation is poorly counteracted by vagal antagonism, whereas during restraint a much higher parasympathetic rebound to sympathoexcitation is observed. In addition, ~70% of the most frequent arrhythmias, i.e., VPB, occurred in the periods of time immediately following attacks, periods which were also characterized by a higher heart rate and a reduced heart rate variability.

In the very first minutes of stress exposure, the sinus tachycardic response was quantitatively similar in the two aversive situations. However, whereas this effect persisted until the end of the test in defeated animals, it was gradually fading out during restraint until it reached baseline values. Correspondingly, the r-MSSD (11, 33), although comparatively low in the very initial phases, was soon (after 3 min) significantly increased during the test in restrained animals but not in defeated ones. The latter result is of particular relevance, because this time-domain parameter has been largely validated as a good measurement of parasympathetic input to the heart (33). Moreover, when the restraint test was performed under muscarinic block (atropine methyl nitrate, 0.5 mg/kg sc) in eight additional age-matched males, the values of r-MSSD were very low (0.6 ± 0.04 ms; unpublished observations; see Table 1 for comparison with the other recording conditions), once more indicating that this parameter is a reliable marker of high-frequency, vagally mediated variations of R-R interval.

A difference between the two stressors in the autonomic regulation of heart rate emerged also when comparisons were made among 15-min mean values of SD, SD/RR, and r-MSSD in the two situations. The SD and the coefficient of variance, which estimate the overall heart rate variability due to the contribution of both branches of the autonomic nervous system, were significantly lower during defeat than during restraint. The variability of adjacent interbeat intervals (r-MSSD) was unchanged during defeat compared with that at baseline but significantly increased during restraint.

The main autonomic response triggered by SS could be a selective sympathoexcitation, an impaired vagal withdrawal, or an increased sympathovagal balance. The high levels of circulating NE suggest that a strong sympathetic activation takes place during SS which is not significantly antagonized by the parasympathetic branch (SD of mean RR is significantly decreased during the test compared with that at baseline, whereas the values of the r-MSSD are unchanged throughout the stress period). On the contrary, restraint is characterized by a significant parasympathetic response to sympathoexcitation (markedly higher values of SD, SD/RR, and r-MSSD compared with baseline), this feature being evident from the third minute of the test onward.

The use of time-domain measurements of heart rate variability as a tool to evaluate the autonomic input to the heart is not entirely free from limitations, especially when applied to short-term recordings. The main limit of these statistical methods is that they provide more qualitative rather than quantitative information. In contrast, power spectral analysis of heart rate variability may provide more detailed information regarding the relative contribution of the two branches of the autonomic nervous system (35). However, time- and frequency-domain measurements are tightly related, i.e., for every frequency-domain measurement there is a time-domain measurement that strongly correlates with it (33). In particular, the r-MSSD is so closely correlated with the high-frequency power of heart rate variability that it is, for all practical purposes, interchangeable with the spectral measure (34). In addition, as pointed out by Lombardi and colleagues (17), despite the impressive growth of research in the field of frequency-domain measurements, the most rewarding clinical results have been so far obtained in the field of prognosis, utilizing the time-domain indexes.

A possible explanation for the different sympathovagal balance between defeat and restraint takes into account the cardiac-somatic relationship (24); i.e., it might result solely from a greater degree of physical exertion in the defeated rats. However, we believe that such an interpretation would be far too restrictive, and
of ventricular abnormalities exhibited by this wild-type strain during defeat was more than three times higher than that observed in Wistar rats exposed to the same social aversive experience (unpublished observations). This finding could be explained in terms of higher sympathetic reactivity to social stress exposure by wild rats compared with Wistar counterparts of the same age. In fact, plasma venous concentrations of catecholamines at the maximum peak (5 min into the test in both strains) were approximately two times higher in wild-type rats than in Wistar rats, although baseline pretest values were fully comparable (27, 29). This consideration is in agreement with data published by Liang and colleagues (16) showing that the levels of circulating catecholamines vary directly with the changes in ventricular vulnerability during psychological stress in dogs. In that study, the substantial rises in blood Epi and NE observed in an angerlike state, indicative of enhanced sympathetic neural activity as well as adrenal medullary discharge, corresponded well with the observed reductions in vulnerable-period threshold. In our study, neuroendocrine and electrocardiographic responses were measured in different groups of animals, which, on one hand, did not allow direct correlations between catecholaminergic and arrhythmia responses but, on the other hand, prevented uncontrollable effects of blood sampling and/or donation maneuvers on hemodynamics and consequently on electrocardiographic measurements (e.g., Banbridge effect and baroreflexes). However, a general association between the level of cardiac sympathetic activation, as indirectly measured by plasma catecholamine measurements, and the amount of rhythm disturbances was clearly found. A social stressor such as defeat, which provoked a much higher incidence of ventricular ectopic beats than a nonsocial aversive event such as restraint, was also characterized by substantially higher plasma catecholamine levels.

In conclusion, the rat autonomic response to the social stressor defeat (at least as far as cardiac electrical activity is concerned) is more sympathetically dominated and more prone to enhance ventricular vulnerability than that produced by the nonsocial stressor restraint. To what extent this difference is due to the autonomic-somatolinear coupling, or rather to different predictability and/or controllability characteristics of the two stressors (15, 23), still remains to be clarified. The application of this experimental model to stress contexts with more controlled physical and psychological contributions could provide more insights in understanding the arrhythmogenic potential of social aversive factors.

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