Cold face test demonstrates parasympathetic cardiac dysfunction in familial dysautonomia

M. J. HILZ,1,2 B. STEMPEL,2 P. SAUER,2 U. HAERTL,2 W. SINGER,2 AND F. B. AXELROD1

1Department of Neurology, New York University, New York, New York 10016; and 2University of Erlangen-Nuremberg, D-91023 Erlangen, Germany

FAMILIAL DYSAUTONOMIA (FD) is a rare, autosomal recessive disorder affecting the development and survival of sensory, sympathetic, and, to a lesser extent, parasympathetic neurons (2, 25). Sympathetic failure is considered the major component of cardiac instability (21, 25, 38, 39), which puts some patients at risk for bradycardia and sudden death. The extent of cardiovagal dysfunc-

1The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
sigh when cold compresses were applied to their face. Respiratory frequency was monitored with a two-belt chest-abdomen inductance plethysmograph after calibration (Respirtrace calibrator; Ambulatory Monitoring, Ardsley, NY).

Systolic, mean, and diastolic blood pressure (BP_sys, BP_mean, and BP_dia, respectively) were continuously recorded from the left radial artery with noninvasive arterial tonometry (Colin Pilot; Colin Medical Instruments, San Antonio, TX) (18). The tonometer consists of an array of 31 equally spaced piezoresistive pressure transducers, an automated positioning system, and signal conditioning and initial calibration by oscillometric cuff measurement of brachial artery blood pressure (18).

Heart rate was monitored as beats per minute with a five-lead electrocardiogram by means of the Colin Pilot monitor. Skin electrodes were positioned at the left and right infracavicular fossa, the anterior iliac crest, and the midsternum.

Skin blood flow was monitored at the first toe pulp with a Periflux laser Doppler (Perimed, Stockholm, Sweden) emitting divergent continuous 780-nm wave light with an intensity =0.8 mW (23). After calibration of the instrument with a motility standard according to the manufacturer, flow was measured in arbitrary perfusion units (PU). Data were sampled at a rate of 32 Hz with commercial recording software (Perisoft; Perimed) and a personal computer (PC) (23).

Spectral analysis of blood pressure and heart rate variability. Blood pressure and heart rate were transferred via analog output into a custom-designed data acquisition and analysis system (HRview; Boston Medical Technologies, Brighton, MA) and a PC. The analog data were digitized by a 3-channel, 12-bit resolution analog-digital converter (PC-LPM-16; National Instruments, Austin, TX). Each channel of data was sampled at 1 kHz and displayed on the PC. For off-line power spectral analysis of BP_sys and heart rate variability, we used the method described by Goldstein et al. (14) and Saul et al. (32). At baseline and during cold face test, analysis time intervals of 64 s of electrocardiogram R-R intervals and BP_sys values were first processed with the interpolation algorithm suggested by Berger et al. (5). This signal was further cleaned of artifact using the splining method described by Albrecht and Cohen (1) and taken for spectral processing with the Blackman-Tukey algorithm described by Berger et al. (6). This resulted in a minimal frequency resolution of 1 cycle/min or 0.0167 Hz. The resampling rate was 4 Hz with a Nyquist frequency of 2 Hz.

Because oscillations do not have fixed periods and center frequency can vary considerably (22), we defined two frequency bands of interest on the basis of preliminary studies (12, 20, 22, 29, 36, 37). The spectral values for blood pressure and heart rate variability were calculated for low (LF; 0.05–0.15 Hz) and high (HF; 0.15–0.5 Hz)-frequency bands. LF-heart rate oscillations are considered to be mediated by combined sympathetic and parasympathetic activity at rest (14, 22, 29, 31, 36, 37), whereas there is a predominance of sympathetic activity during stressful conditions (14, 31). HF heart rate oscillations are associated with respiratory sinus arrhythmia and reflect parasympathetic activity (14, 22, 29, 31, 36, 37). To quantify LF and HF modulations, the integral under the power spectral density (beats·min⁻²·Hz⁻¹) curve of heart rate was computed for the two frequency bands and expressed as LF and HF power (beats/min²) (14, 29).

To assess baroreceptor reflex sensitivity before and during cold face test, we calculated the gain of the transfer function between PB_sys and heart rate in the LF (0.05–0.15 Hz) band for coherence values >0.5 (30, 32, 38).

Time domain analysis of heart rate variability. The following time domain parameters were calculated from the R-R interval tachogram at baseline and during cold face test: the root mean square successive differences (RMSSD) as a measure of parasympathetic activity (36, 37) and the SD and coefficient of variation (CV), both reflecting sympathetic and parasympathetic heart rate modulation (36, 37).

Calculation of cold face test-to-baseline ratios. Baseline values were obtained by recordings of heart rate, blood pressure, and skin blood flow during a 60-s interval that ended 30 s before the actual cold face test to assure that baseline values were not biased by preparatory activities. The measurements for each parameter were averaged.

To adjust for baseline differences between patients and controls, values during stimulation were related to the corresponding baseline values, and cold face test-to-baseline ratios were calculated for every second of cold face test (15, 19).

To assess the differences between results of patients and controls, repeated-measures ANOVAs were performed on heart rate, blood pressure, respiration, and skin blood flow. The values measured at baseline and during the 60 s of cold face test were used as within-subject factors, and the group to which the participants belonged, i.e., FD patients or control persons, was used as a between-subject factor. The repeated-measures ANOVA was performed on relative values, i.e., cold face test-to-baseline ratios, as well as on absolute values.

To test for overall changes induced by cold face test, the 60-s mean heart rate, blood pressure, and skin blood flow values averaged during stimulation were calculated for each participant, and the percentage change from baseline was evaluated (15, 19). Differences between cold face test and baseline values were analyzed for the patient and the control group with a two-sided Wilcoxon test. Differences between patient and control values during cold face test were evaluated by Mann-Whitney U test.

To identify the onset and persistence of significant heart rate, blood pressure or skin blood flow changes during cold face test in patients and controls, heart rate, blood pressure, and skin blood flow ratios obtained after 5, 10, 20, 30, 40, 50, and 60 s of stimulation were compared with the baseline with a two-sided Wilcoxon test. In RESULTS and Table 1 data are presented as means ± SD.

A commercially available statistical program (SYSTAT, Evanston, IL) was used for statistical calculations. The level of significance was set at P ≤ 0.05.

RESULTS

Cold stimulation was perceived immediately by all patients and controls, and all reported the 60-s stimulation as similarly unpleasant. On the numerical visual analog scale, patients and controls rated the discomfort from 6 to 9. The Mann-Whitney U test showed no difference between ratings of patients (mean 7.7 ± 1.1) and controls (mean 7.3 ± 0.9).

Directly measured baseline values. At baseline, patients with FD had significantly higher blood pressures and heart rates than controls (Mann-Whitney U test, P < 0.05). For FD patients, BP_dia was 79.3 ± 18.4 mmHg, BP_mean was 96.7 ± 18.9 mmHg, BP_sys was 131.4 ± 20.6 mmHg, and mean heart rate was 77.9 ± 11.7. In contrast, for control persons BP_dia was 56.4 ± 7.8 mmHg, BP_mean was 73.1 ± 7.4 mmHg, BP_sys was 106.5 ± 9.2 mmHg, and mean heart rate was 67.2 ± 11.9 beats/min. Baseline skin blood flow was not significantly different, 49.3 ± 53.2 PU in patients and 46.7 ±
50.1 PU in controls, nor was respiratory frequency, 18.1 ± 5.1 cycles/min (cpm) in patients and 17.1 ± 4.5 cpm in controls.

Measured responses to cold face test. In patients with FD, cold face test did not cause any sustained significant change of heart rate or blood pressure within the stimulated 60-s period (Figs.1 and 2). In the first 5 s of cold face test, heart rate significantly decreased by 6.0 ± 6.3% (Wilcoxon, P < 0.05), but there was no significant heart rate change from baseline noted at the other measured intervals (10, 20, 30, 40, 50, and 60 s). Skin blood flow showed a slight but not significant decrease after 4 s of cold face stimulation, reaching a nadir after 6 s with a decrease of 8.7 ± 15.3%. Skin blood flow returned to baseline values in all patients by 13 s.

In controls, changes in blood pressure, skin blood flow, and heart rate were noted within 5 s of cold face stimulation. Blood pressure began to rise immediately, and within 5 s values were significantly different from baseline (Wilcoxon, P < 0.05). Blood pressure increased continuously during the stimulation period, with an average rise of 9.9 ± 7.7% (Table 1; Fig. 2). Skin blood flow started to decrease immediately, and within 5 s values were significantly different from baseline (Wilcoxon, P < 0.05). During the stimulation period, there was an average reduction of skin blood flow of 35.8 ± 28.5%. There was a great variability in the time it took a particular individual to reach a nadir for flow, with a range of 3–21 s (mean 8.6 ± 5.2 s). Skin blood flow steadily returned to baseline in controls so that by the end of the stimulation period values did not differ from baseline (Wilcoxon, P = 0.221; Fig. 3).

In contrast to the patients with FD, cold stimulation did decrease the heart rate in controls, but the responses were slower and more sustained than the blood pressure and skin blood flow responses. At 5 s, the heart
rate had slowed by 2.3 ± 2.0%, which was not significant. The slowing was significant for values taken at 10, 20, 30, 40, 50, and 60 s. The average slowing over the 60-s test period was 5.4 ± 6.7%. The time it took a particular individual to reach the nadir for heart rate was 24–36 s, with an average maximum decrease of 19.1 ± 9.8%. After reaching the nadir, heart rate steadily increased despite continuing cold stimulation. However, heart rate did not return to baseline values within the 60 s of cold stimulation.

Respiratory frequency during baseline did not differ between controls (17.1 ± 4.5 cpm) and patients (18.1 ± 5.1 cpm) (Mann-Whitney, P > 0.05) and did not change significantly during cold face test in controls (16.6 ± 4.6 cpm) and FD patients (19.1 ± 3.8 cpm) (Wilcoxon, P > 0.05).

Repeated-measure ANOVA showed significant changes with cold face test for the main effect group, as well as for the main effects of skin blood flow, heart rate, and BPsys, BPdia, and BPmean (P < 0.05), but not for the main effect of respiration.

Time domain measures of heart rate variability at baseline, RMSSD and SD of heart rate variability were lower in patients than in controls (Mann-Whitney U test, P < 0.05; see Table 1). During stimulation, RMSSD, SD, and CV increased significantly in controls (Wilcoxon, P < 0.05) but did not change in FD patients.

Power spectral analysis of baseline heart rate variability showed less HF activity in FD patients (3.3 ± 3.4 beats/min²) than in controls (12.0 ± 17.7 beats/min²; Mann-Whitney U test, P < 0.05; see Table 1), whereas LF activity showed no significant difference between patients (8.4 ± 7.1 beats/min²) and controls (7.6 ± 6.4 beats/min²; Fig. 4).

Power spectral analysis of heart rate variability during cold face test documented a significant increase of HF power (18.1 ± 26.4 beats/min²) but no change of

---

### Table 1. HR, BP, and SBF changes during CFT and time and frequency domain parameters at baseline and during CFT

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 15)</th>
<th>Wilcoxon Test P Values</th>
<th>FD Patients (n = 11)</th>
<th>Wilcoxon Test P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR decrease after 5 s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>of CFT, %</td>
<td>2.3 ± 2.0</td>
<td>&gt;0.05</td>
<td>6.0 ± 6.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HR decrease during 60 s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>of CFT, %</td>
<td>5.4 ± 6.7</td>
<td>&lt;0.05</td>
<td>0.8 ± 5.3</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>BP increase during 60 s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>of CFT, %</td>
<td>9.9 ± 7.7</td>
<td>&lt;0.05</td>
<td>0.1 ± 7.4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>SBF decrease during 60 s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>of CFT, %</td>
<td>35.8 ± 28.5</td>
<td>&lt;0.05</td>
<td>4.9 ± 16.2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>RMSSD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>74.0 ± 53.6</td>
<td>29.1 ± 14.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>During 60 s of CFT</td>
<td>100.8 ± 70.4</td>
<td>&lt;0.05</td>
<td>27.9 ± 13.5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>CV, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>7.1 ± 3.6</td>
<td>5.0 ± 2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>During 60 s of CFT</td>
<td>8.9 ± 3.7</td>
<td>&lt;0.05</td>
<td>6.1 ± 2.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.07 ± 0.04</td>
<td>0.04 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>During 60 s of CFT</td>
<td>0.08 ± 0.04</td>
<td>&lt;0.05</td>
<td>0.05 ± 0.01</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>HF power, bpm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>12.0 ± 17.7</td>
<td>3.3 ± 3.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>During 60 s of CFT</td>
<td>18.1 ± 26.4</td>
<td>&lt;0.05</td>
<td>3.5 ± 3.4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>LF power, bpm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>7.6 ± 6.4</td>
<td>8.4 ± 7.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>During 60 s of CFT</td>
<td>7.9 ± 4.0</td>
<td>&gt;0.05</td>
<td>6.9 ± 8.0</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of patients. Heart rate (HR), blood pressure (BP), and skin blood flow (SBF) are expressed as percent change from baseline. CFT, Cold face test; RMSSD, root mean square of successive differences; CV, coefficient of variation; HF, high frequency; LF, low frequency; bpm, beats per minute.

---

Fig. 3. Exemplary course of skin blood flow (SBF) in male, 24-yr-old FD patient and in male, 27-yr-old control person at BL, 30 s before CFT, and during 60 s of CFT. SBF values are measured in arbitrary perfusion units (PU). SBF decreased significantly in control person and showed no significant decrease in FD patients.
Cold face test assesses the integrity of the trigeminal-brain stem vagal reflex arc. In contrast to other autonomic challenge maneuvers, such as paced breathing or Valsalva test, cold face test does not require active patient participation. In the cold face test, the bradycardiac response is induced by cold, wet, or noxious stimulation of the face (19, 24) via reflex centers located in the medullary region (19). Efferent sympathetic pathways mediate peripheral vasoconstriction (15, 16) and secondary blood pressure increase (12, 15, 19). Efferent parasympathetic pathways mediate bradycardia (19), which can be abolished by atropinization or vagotomy (10, 16, 19). A disturbance of the integrity of the trigeminal-brain stem vagal reflex arc at any level yields an abnormal cold face test response with absent or diminished bradycardia (19).

Because the number of temperature-mediating small nerve fibers is reduced in FD (4, 26, 27), one might doubt the feasibility of adequate afferent stimulation with cold face test in FD patients. Temperature, like pain appreciation, is not totally absent in FD, so that if a sufficiently large area is stimulated, the sensation or stimulus will be perceived. Thus all FD patients did perceive the ice stimulation as highly unpleasant, with negative reactions that often surpassed those of our controls. In a pilot study with five FD patients, it was found that cold face test could not be tolerated for the planned 2 min because all five patients demanded to abort ice stimulation within 75–80 s. Therefore, cold face test was limited to 60 s in this study. Obviously, the 0–1°C cold stimulus was still sufficient to induce an immediate and intense sensation in the patients. Sudden temperature changes from normal, “neutral” skin temperature to 0–1°C induce HF firing bursts in nociceptors and temperature-mediating fibers (7). The ratings of thermal perception were not lower in the patients than the controls. Although the self-report of the cold sensation does not quantify the extent to which sensory fibers were stimulated, the ratings and the discomfort reported by the patients suggest that the afferent branch of the cold face test reflex arc was sufficiently stimulated. We assume that abrupt exposure to the ice-cold compresses induced a temporal impulse summation that compensated for the reduced spatial summation caused by small fiber reduction in FD patients (7).

Therefore, we assume that the reduced sympathetic and parasympathetic responses of patients are not caused by inadequate afferent stimulation but by deficiencies in brain stem centers or in the efferent sympathetic and parasympathetic branches of the reflex arc.

Our control subjects exhibited responses that were similar to those reported in previous studies (12, 15, 16, 19, 20). They had a cardiovagal response with a maximum bradycardia after 24–36 s (15, 20) and a sympathetic response with an immediate onset of skin blood flow decrease and blood pressure increase in response to the initial nociception (17). Theoretically, bradycardia might be mediated by changes of the respiratory pattern or the baroreceptor activity secondary to blood pressure increase. However, respiration did not change during cold face test in either group. Therefore, heart rate changes cannot be attributed to altered respiration. Baroreflex activation also had no major influence on heart rate responses during stimulation. In the controls, the LF transfer function gain between BP_{sys} and heart rate increased during cold stimulation, indi-
cating a higher baroreceptor sensitivity. The increase in gain between blood pressure and heart rate indicates that a predetermined blood pressure change resulted in a greater change of heart rate during the maneuver than before stimulation (11). Only if baroreflex characteristics changed with cold face stimulation, i.e., if thresholds reset or the firing rate hysteresis was modified, could the additional decrease of heart rate be ascribed to baroreflex activation. Major changes of these baroreflex properties are unlikely. Therefore, we assume that bradycardia during cold face stimulation is the result of an additional cardiovascular activation during cold face test. This assumption is in conformity with several studies that showed that baroreflex activity is not essential to cold face test-induced bradycardia, inasmuch as heart rate decrease can be seen with complete absence of blood pressure changes (10, 16). Moreover, the FD patients showed an early, although significantly diminished, bradycardia, whereas blood pressure did not change at all in the early phase of stimulation. Therefore, it is unlikely that bradycardia resulted from baroreflex activation.

In the patients, the period of vasoconstriction was limited, brief, and inadequate to induce any blood pressure changes. This indicates inadequate sympathetic outflow, which is a common finding in FD (2, 39). Sympathetic failure can be attributed to a reduction of peripheral sympathetic fibers and neuronal somas in cervical and thoracic sympathetic ganglia to 27–37% of the normal number (26–28).

In addition to the sympathetic dysfunction, the parasympathetic response to cold face stimulation was also inadequate in the patients. Bradycardia was much less pronounced in the FD patients than in the controls. In FD patients, heart rate decreased 6.0 ± 6.3%, whereas in controls, heart rate decreased 19.1 ± 9.8%. The defect of parasympathetic activation (22, 36, 37) is further supported by the lack of RMSSD or HF power increase in the FD patients.

Parasympathetic dysfunction has not yet been clearly defined (21). Smith et al. (33) observed miosis and tearing in FD patients after local instillation of dilute methacholine and concluded that there is a deficiency of parasympathetic innervation accounting for the supersensitive responses of pupils and lacrimal glands. With intravenous administration of methacholine, Smith and colleagues (34) also reported enhanced parasympathetic responses such as exaggerated tearing, coughing, gut activity, and fall of blood pressure without compensatory increase of heart rate. Again, the authors assumed that there is an insufficiency of parasympathetic function with effector supersensitivity.

Maayan et al. (21) report inadequate modulation of parasympathetic activity in FD patients with abnormal retention of parasympathetic heart rate variability on standing. Our results confirm the previous findings and also show inadequate parasympathetic response. In contrast to standing, patients should increase cardiovagal activity with cold face stimulation.

Our data suggest that the deficient increase of cardiovagal activity during cold face stimulation reflects inadequate central or peripheral cardiovagal modulation. The dysfunction might originate from brain stem levels of the cold face test reflex arc, inasmuch as abnormalities of brain stem areas related to parasympathetic modulation have been described in FD patients (13, 25). Pearson et al. (25) observed spongy changes in the central tegmental tract that account for dysfunctions of the brain stem reticular formation, such as blood pressure and respiratory control. Gadodt et al. (13) recorded abnormal respiratory patterns and absence of parasympathetically mediated respiratory heart rate variability in all their FD patients. The authors described brain stem respiratory “disconnections” that involve the vagus nerve (13).

In summary, cold face test demonstrated a reduction of parasympathetic cardiac modulation in FD patients. The impaired brain stem-cardiovascular reflex provides further support for the assumption that central abnormalities contribute to the cardiovascular instability and risk of arrhythmias in FD patients.

Perspectives

The results of our study confirm that cold face test can be used to assess cardiovagal modulation without stimulation of baroreceptors (10, 15, 16). Further studies, including baroreceptor stimulation by means of neck suction or phenylephrine infusion, might enhance the understanding of parasympathetic regulation in familial dysautonomia (9, 35) and show whether the afferent branch of the baroreflex arc contributes to a reduced modulation of cardiovagal activity. Although stimulus standardization might be more demanding with the cold face test than with neck suction or pharmaceutical baroreceptor stimulation, a combination of both approaches seems to be suited for the differentiation of parasympathetic dysfunctions. Abnormal results of cold face stimulation with normal findings of baroreceptor stimulation suggest a central or efferent dysfunction of cardiovagal stimulation. The opposite findings are more indicative of an afferent baroreceptor dysfunction. Thus cold face test not only refines the pathophysiological knowledge of familial dysautonomia but might further the understanding of more widespread diseases such as diabetic autonomic neuropathy.

The authors are grateful to Luciano Bernardi (Univ. of Pavia, Italy) for comments and suggestions regarding this paper and to E. Pauli for advice and help with the statistical analysis of our data.

The study was partially funded by the Dysautonomia Foundation (New York, NY).

Address for reprint requests and other correspondence: M. J. Hilz, Dept. of Neurology, New York Univ. Medical Center, 550 First Ave., Suite NB 7W 11, New York, NY 10016.

Received 3 April 1998; accepted in final form 2 March 1999.

REFERENCES


