Enhanced Affective Startle Modulation in Salt-Sensitive Subjects

Konrad Buchholz, Hartmut Schächinger, Miriam Wagner, Ulrike Schorr, Arya Mitra Sharma, Hans Christian Deter

Abstract—Salt-sensitive normotensive men exhibit an enhanced pressor response to mental stress. Although an enhanced pressor response is associated with higher affective startle modulation in men, an association between salt sensitivity of blood pressure and affective startle modulation has not been studied so far. We studied reactivity to mental stress and startle modulation in 14 salt-sensitive healthy white male students and 14 salt-resistant control subjects, who were well matched for age, body mass index, physical fitness, and family history of hypertension. Subjects performed a computerized information-processing task under time pressure (manometer test), while heart rate and blood pressure were continuously registered. In a separate session, subjects viewed a series of 42 pictures of the International Affective Picture System (IAPS), varying in pleasure and arousal, while acoustic startle probes were administered randomly, and electromyogram activity of the orbicular eye muscle was continuously recorded. Startle modulation was calculated as the difference between startle responses under negative and positive affective stimuli. In contrast to salt-resistant subjects, salt-sensitive subjects showed significantly enhanced startle amplitudes under negative stimuli and diminished amplitudes under positive stimuli. Thus, salt-sensitive subjects displayed a significantly higher startle modulation than did salt-resistant subjects ($P<0.05$). Subjective ratings of the presented IAPS pictures did not differ between the groups. The increased startle modulation of salt-sensitive subjects suggests an enhanced activity of the central nucleus of the amygdala. This enhanced central nervous responsiveness may contribute to higher sympathetic pressor reactivity and, thus, to the later development of hypertension in salt-sensitive individuals. (Hypertension. 2001;38:1325-1329.)

Key Words: startle modulation ▪ stress ▪ normotension ▪ hypertension, sodium-dependent ▪ blood pressure

In most, but not all, studies, an enhanced cardiovascular reactivity was found in hypertensive patients, borderline hypertensive patients, and healthy offspring of hypertensive parents, and it has been hypothesized that an enhanced cardiovascular reactivity in healthy volunteers may contribute to the later development of hypertension. Increased pressor response has also been described as a consequence of salt loading in a proportion of young normotensive individuals, and several lines of evidence suggest that these salt-sensitive individuals may be genetically prone to the development of hypertension. Salt sensitivity was shown to predict future hypertension, and an enhanced noradrenergic response was found in normotensive salt-sensitive subjects. We have previously shown that young normotensive salt-sensitive subjects also display an elevated blood pressure and heart rate response to mental stress.

However, it is not clear whether the enhanced reactivity of salt-sensitive subjects originates in peripheral factors, such as an impaired regulation of adrenoreceptor sensitivity, endothelial factors, or volume expansion, or through central nervous factors, as suggested by the enhanced anxiety and irritability observed in salt-sensitive subjects.

On the basis of studies in animal models, tools for the study of central mechanisms, including the startle response to threat and danger, have been developed for use in human studies. In a recent study, Gautier and Cook observed an association between startle modulation under negative versus positive affective conditions and cardiovascular reactivity to mental stress. In their study, high startle modulators displayed a higher systolic stress response than did intermediate and low startle modulators. Therefore, the aim of our present study was to examine the hypothesis that salt sensitivity of blood pressure may also be associated with an enhanced affective startle modulation.

Methods

Subjects
Of 31 healthy white male students (age, 20 to 30 years) who volunteered for the study, 14 salt-sensitive and 14 salt-resistant subjects were matched by participant for age (±1 year), body mass index, physical fitness, and family history of hypertension.
index (±1.5 kg/m²), and physical activity (±1 degree in a tripartite categorization). Three salt-resistant subjects were excluded from analysis, because <50% of their startle trials could be scored by the criteria described below.13 All subjects underwent routine physical examination, laboratory evaluation, and phenotypic characterization regarding their blood pressure response to a dietary salt-loading/restriction protocol described elsewhere.14,15

To assess parental history of hypertension, written information was obtained from the parents’ family physicians. Subjects with at least 1 hypertensive parent were regarded as having a positive family history of hypertension.

Study Protocol

During the psychophysiological tests, blood pressure was continuously registered by Finapres (Ohmeda). Heart rate was continuously registered by ECG. During the startle experiment, orbicularis oculi muscle activity was measured by electromyogram (EMG). The raw signal of the EMG was amplified and rectified with a root-meansquare rectifier and integrator (time constant, 10 milliseconds).16

One to 3 days before the salt-loading/restriction protocol began, a computerized information processing task, the Manometer Test,17 was performed as described elsewhere.6 The test was shown to elicit reproducible blood pressure and heart rate responses that are within the expected range of comparable mental stress tests.18-20

The startle experiment, which was similar to that of Bradley et al,21 was conducted after completion of the salt-loading/restriction protocol under normal sodium balance. Subjects were presented with a sequence of 42 pictures, representing positive, neutral, and negative valence categories (see Appendix). The assignment of pictures to valence and arousal categories was based on original International Affective Picture System (IAPS) ratings.22,23 The presentation was divided into 3 blocks of 14 pictures, given in a randomized order with a counterbalanced serial position of an equal number of valence conditions in each block to enhance unpredictability. Each picture was presented for 6 seconds with a dark period of 14 seconds between the pictures. Pictures were shown on a 19-inch PC screen at a viewing distance of ~75 cm in a darkened room. Subjects were instructed to view pictures for the whole presentation period. No other instructions were given.

Standard acoustic noise stimuli (noise generator provided by Dr I. Curio, Medizinelektronik, Bonn, FRG; impulse duration, 50 milliseconds; 95 dB; instantaneous rise time) were presented binaurally via monaural headphones. The probability of noise stimuli during seconds; 95 dB; instantaneous rise time) were presented binaurally by Dr I. Curio, Medizinelektronik, Bonn, FRG; impulse duration, 50 milliseconds; 95 dB; instantaneous rise time) were presented binaurally.

The protocol of the present study was approved by the ethics committee of our hospital. All subjects gave informed consent before participation.

Data Analysis

All physiological signals were continuously recorded at 1000 Hz via DOS software (Turbolab, Stemmer). The ECG, Finapres, and EMG recordings were analyzed offline under visual artifact control. Blood pressure and heart rate responses to the mental task were determined by subtracting the average of the readings during baseline and test periods. Startle blink amplitude was calculated as the difference between the maximum of the rectified EMG signal in a time window of 20 to 150 seconds after startle stimulus presentation and the median of the 50 milliseconds before the upstroke of the blink response.13 Blink responses were coded as missing if the EMG fluctuated by >30% of the blink amplitude during 50 seconds before the onset of the blink response and if no typical blink response was detectable (together, <5% of the trials).13

EMG amplitudes of the startle reactions were logarithmically transformed. The z score was then related to individual dark startle mean and variability. The affective startle modulation index was calculated by subtracting the average of the EMG amplitudes during the 9 positive pictures from the average of the EMG amplitudes during the 9 negative pictures.

To assess cardiovascular reactions to the startle presentation, beat-to-beat values of interbeat intervals and systolic and diastolic blood pressure were adjusted to values per second. Difference values to baseline (just at the point of the startle presentation) were computed for each 11 seconds that followed the startle presentation.

Statistical Analysis

Statistical analysis was performed by SPSS+ (SPSS Inc). All data are presented as mean±SD. Comparisons between salt-sensitive and salt-resistant subjects and comparisons of changes within groups were performed by Wilcoxon tests. For comparisons between startle modulation groups, Mann-Whitney U tests were used. A value of P<0.05 was considered statistically significant. Additionally, an ANOVA for repeated measures with the respective valence condition as the repeated factor was performed.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Results

Baseline characteristics of salt-sensitive and salt-resistant subjects are shown in Table 1. Subjects were comparable with respect to age, body mass index, physical fitness, and family history of hypertension. By definition, the difference in mean arterial blood pressure between the high- and low-salt periods was greater in the salt-sensitive than in the salt-resistant group.

Baseline levels of systolic (115.79±17.10 versus 108.43±8.91 mm Hg) and diastolic (63.79±9.44 versus 59.57±5.09 mm Hg) blood pressure before the mental stress test were not significantly different between salt-sensitive versus salt-resistant subjects, respectively, but salt-sensitive subjects, compared with salt-resistant subjects, displayed greater heart rate (10.02±9.44 versus 4.97±7.04 bpm, respectively; P<0.01) and systolic responses (19.45±9.08 versus 13.77±6.15 mm Hg, respectively; P<0.02) (Figure 1) and tended to display greater diastolic responses (10.37±3.22 versus 8.38±3.89 mm Hg, respectively; P<0.10).

ANOVA of startle responses revealed a significant within-subject effect of valence category (P<0.02) and a significant interaction between valence categories and the status of salt sensitivity (P<0.05). Subsequent analysis showed that salt-
sensitive subjects displayed significantly higher startle blink amplitudes during negative versus positive affective pictures ($P<0.01$, Table 2). Startle amplitudes during negative and neutral pictures were higher in salt-sensitive subjects ($P=0.02$ and $P<0.05$ for logarithmically transformed and $z$-scored values, Table 2). Startle modulation, ie, the difference between startle amplitudes during negative and positive pictures, was significantly greater in salt-sensitive than in salt-resistant subjects ($P<0.05$, Figure 2). Self-assessment Manikin ratings on affective valence contents of the pictures showed that salt-sensitive and salt-resistant subjects judged the pictures according to the given valence categories by the IAPS and that there were no differences between groups (Table 2).

Analysis of mean interbeat interval and systolic and diastolic blood pressure reactions over 11 seconds after the dark startles yielded no significant differences between salt-sensitive and salt-resistant subjects.

TABLE 2. Startle Blink Amplitudes and Subjective Ratings According to Valence Categories of Picture Stimuli in Salt-Sensitive and Salt-Resistant Subjects

<table>
<thead>
<tr>
<th>Valence Category</th>
<th>Salt-Sensitive Subjects ($n=14$)</th>
<th>Salt-Resistant Subjects ($n=14$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valence ratings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive IAPS</td>
<td>7.12 ± 0.73†</td>
<td>7.30 ± 0.63†</td>
</tr>
<tr>
<td>Negative IAPS</td>
<td>2.58 ± 0.66†</td>
<td>2.35 ± 0.58†</td>
</tr>
<tr>
<td>Neutral IAPS</td>
<td>5.37 ± 0.34†</td>
<td>5.57 ± 0.60†</td>
</tr>
<tr>
<td>Startle blink amplitude, $\mu V$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive IAPS</td>
<td>168.19 ± 167.17*</td>
<td>196.66 ± 207.32</td>
</tr>
<tr>
<td>Negative IAPS</td>
<td>227.65 ± 223.68*</td>
<td>211.94 ± 235.65§</td>
</tr>
<tr>
<td>Neutral IAPS</td>
<td>199.49 ± 182.64*</td>
<td>222.52 ± 271.56§</td>
</tr>
<tr>
<td>Dark period</td>
<td>201.35 ± 205.52*</td>
<td>216.47 ± 242.07</td>
</tr>
</tbody>
</table>

Values are mean±SD. Valence ratings were collected by using Self-Assessment Manikin scales ranging from 1 to 9 (1 indicates extremely negative; 5, neutral; and 9, extremely positive).

Correlational analysis of an association of startle blink response and cardiovascular reactivity revealed a nearly significant correlation between startle modulation and systolic blood pressure increase under mental stress (Pearson correlation $r=0.31$, $P=0.05$). When the cohort was subdivided into groups of high, intermediate, and low startle modulators, intermediate startle modulators showed a significantly higher systolic increase under mental stress than did low startle modulators (18.44±6.70 versus 12.56±5.90 mm Hg, respectively; $P<0.05$), and high startle modulators showed a trend to a higher systolic increase than did low startle modulators (18.64±10.55 versus 12.56±5.90 mm Hg, respectively; $P<0.10$).

There were no significant differences between salt-sensitive and salt-resistant subjects in the following psychometric variables: trait anxiety, which was collected with use of the State Trait Anxiety Inventory ($25$ (32.43±6.26 versus 34.93±7.45, respectively), and emotional irritation, which was collected with use of the Adjective Word List (EWL 60 S) ($26$ (5.93±6.02 versus 5.57±4.01, respectively).

Discussion

In a previous study, we have shown that salt-sensitive normotensive subjects display an increased pressor and heart rate response to mental stress. In the present study, we extend these findings by demonstrating that these subjects also display an enhanced affective modulation of the startle-induced blink responses during presentation of standardized pictures selected from the IAPS. Thus, the present study is the first to demonstrate that central factors, which are involved in affective modulation, may play a role in the blood pressure response to changes in dietary salt intake and perhaps contribute to the development of hypertension in salt-sensitive normotensive individuals.

The modulation of startle-induced blink reactions during appetitive and aversive affective states is believed to reflect the modulating influence of the amygdaloid body on emotional reflex autonomic and somatomotor functions. Projections of the central nucleus of the amygdala to autonomic...
circulatory centers in the brain stem\textsuperscript{29–31} suggest a link between an individual’s neurophysiological disposition to reactions to threat and the regulation of blood pressure and heart rate. Therefore, it is not surprising that an auditory startle signal, as used in the present study, has been shown to elicit acute blood pressure and heart rate responses, which are mediated by sympathetic activation.\textsuperscript{32} The hypothesis of a link between affective modulation of the startle response and the modulation of the cardiovascular response to mental stress is also in line with the previous observation that subjects with a higher affective startle modulation display an enhanced reactivity to mental stress.\textsuperscript{12} The latter observation is replicated similarly by the finding of a correlation between startle modulation and systolic blood pressure reactivity to mental stress in the present study.

Our present finding of an enhanced startle modulation in salt-sensitive subjects may, in part, explain our previous observation of an enhanced blood pressure reactivity to mental stress in these subjects.\textsuperscript{6} In previous studies, we have observed that subjects usually report distress or negative affective engagement during mental stress. This is clearly compatible with the suggestion by Gautier and Cook\textsuperscript{33} that mental stress, like other aversive stimuli in some,\textsuperscript{33} but not all,\textsuperscript{31} experimental animals, potentiates the output from the central nucleus of the amygdala. Therefore, we speculate that the enhanced negative affect may enhance the neurovegetative output from the amygdala and thus act as an important contributor to the increased pressor and chronotropic response to mental stress in salt-sensitive subjects.

Interestingly, on the basis of our present findings in salt-sensitive individuals, we may perhaps also speculate that enhanced output of the central nucleus of the amygdala together with the increase in sympathetic response to negative affective stimuli may play a role in the development of salt sensitivity. This hypothesis would be well in line with previous observations by Light and colleagues,\textsuperscript{34,35} who demonstrated a decrease in natriuresis during mental stress in subjects genetically predisposed to the development of hypertension. Furthermore, psychosocial stress, as induced by preparation for exams, has been shown to increase the occurrence of a salt-sensitive response to dietary salt intake in medical students.\textsuperscript{36} This sodium-retaining or antinatriuretic effect of mental stress is apparently mediated by increased activity of renal sympathetic nerves.\textsuperscript{37} Thus, it may well be that the enhanced sympathetic response observed in our salt-sensitive subjects may be driven by an enhanced innate sensitivity of the phylogenetically older parts of the emotional system, making them more vulnerable for mental and emotional challenges in daily life.

Contrary to the observed eye-blink reaction differences between salt-sensitive and salt-resistant subjects, no different cardiovascular reactions to the startle presentation were found. This null result may be due to the small sample size in the present study. Further studies on cardiovascular reactions to startle probes of short duration are needed.

In contrast to previous findings,\textsuperscript{10} the present study revealed no differences in anxiety or irritability between salt-sensitive and salt-resistant subjects.

Although we studied salt-sensitive and salt-resistant individuals, it is important to note that we did not examine the effect of salt intake on psychophysiological parameters in the present study. That issue has previously been addressed by other investigators, albeit with inconsistent results. Thus, although Staessen et al\textsuperscript{38} found an interaction between a high-salt intake and work-related stress in 348 male civil servants, others have failed to demonstrate an effect of dietary salt loading on the blood pressure response to mental arithmetic in young normotensive or hypertensive adults.\textsuperscript{39} It should also be noted that our findings are restricted to men.

In summary, in the present study, we provide evidence for an enhanced modulation of the startle response to affective changes in salt-sensitive subjects. This finding may, in part, explain our previous observation of an increased pressor response to mental stress in salt-sensitive men. Enhanced startle modulation may reflect increased excitability of the amygdala of salt-sensitive men.

**Appendix**

The IAPS numbers are as follows: for positive pictures, 1710*, 5787, 8200*, 7580*, 2030, 4250*, 1750*, 5626, 7230*, 2340, 2160*, 4180*, and 1600*; for neutral pictures, 7550*, 7004, 7010*, 7130*, 7182, 9070*, 7830*, 7090*, 7100*, 5740, 2190*, and 7190*; and for negative pictures, 2682, 6360*, 6550, 6230*, 2800*, 6540*, 2661, 2661, 9570, 4621, 1120*, 9140*, 1300*, 2750, 2120*, 9600, 3030*, and 6821. An asterisk denotes pictures that were probed with a startle stimulus. Pictures were given in randomized order of blocks A, B, and C, and the order given here is ABC.

**Acknowledgment**

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**References**

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