Effect of Cold Pressor Test–Induced Stress on Leukocyte Sodium Transport and Norepinephrine

ANGELINA RIOZZI, ANTHONY M. HEAGERTY, ROBERT F. BING, HERBERT THURSTON, AND JOHN D. SWALES

SUMMARY The effects of stress on leukocyte membrane sodium efflux rate constant and plasma norepinephrine levels were studied before and during cold pressor test in normotensive subjects with and without a family history of hypertension. After 20 minutes of supine rest, no significant differences in total, ouabain-resistant or ouabain-sensitive sodium efflux rate constants were apparent between the two groups. In normotensive subjects with no family history, there was no significant change in any efflux rate constant during cold pressor test, although there was a highly significant negative correlation between change in total efflux rate constant and change in norepinephrine levels ($r = -0.82$, $p< 0.01$, $n = 12$). During cold pressor test in subjects with a family history of hypertension, there was a significant rise in the ouabain-resistant efflux rate constant ($1.5 \pm 0.1$ vs $1.0 \pm 0.1$ hr$^{-1}$; $p<0.01$, $n = 10$); this level was also significantly higher than that in control subjects ($p<0.002$). In this group, the ouabain-sensitive efflux rate constant fell slightly but not significantly ($1.8 \pm 0.2$ vs $2.1 \pm 0.2$ hr$^{-1}$; $n = 10$). These results suggest that stress in the form of a cold stimulus produces qualitative differences in leukocyte cation transport in normotensive offspring of hypertensive patients as compared with subjects without such a family history. Hypertension 9:13–17, 1987

KEY WORDS • stress • hypertension • leukocyte sodium transport

NUMEROUS reports describe alterations in cation handling by cells obtained from patients with essential hypertension and from spontaneously hypertensive rats. One abnormality has been found consistently: in white blood cells of essential hypertensive patients plasma membrane Na$^+$, K$^+$-adenosine triphosphatase pump activity is depressed, as measured by the ouabain-sensitive efflux rate constant for sodium. This finding has become central to a hypothesis relating sodium efflux to the cellular mechanisms that generate raised blood pressure by postulating that reduced Na$^+$-K$^+$ pump activity is due to humoral inhibition. However, depression of leukocyte ouabain-sensitive sodium efflux has also been reported in the normotensive first-degree relatives of essential hypertensive patients, thereby dissociating this phenomenon from blood pressure elevation per se.

In addition, this theory fails to explain disturbances in other sodium transport systems. An alternative hypothesis proposes that these findings are manifestations of a genetically determined alteration of the physicochemical structure of the cell membrane. In this regard it has been shown that bone marrow–transplanted rats display the cation transport characteristics of the donor animal, and many of the other abnormalities of univalent and divalent ion transport initially reported in established human essential hypertension have now been found in normotensive offspring of such patients. In addition, a recent investigation has demonstrated differences in the pattern of response in leukocyte sodium transport, with alterations of sodium balance in subjects with and without a family history of raised blood pressure. The role of catecholamines and stress in the genesis of hypertension is also controversial; nevertheless, in the early phases of hypertension, plasma catecholamine levels are thought to be raised. Therefore, we decided to investigate whether the induction of stress could affect leukocyte sodium transport and whether the effects would differ between subjects with and subjects without a family history of hypertension.

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Materials and Methods

Twenty-two normotensive white volunteers were studied, 10 of whom had at least one parent known to have essential hypertension (+ FH). The diagnosis of hypertension was confirmed from hospital records in five patients; three of the five had sustained myocardial infarction and the other two had had cerebrovascular accidents. Four of the remaining volunteers were medical students and measured the blood pressure of their relatives. One volunteer was not a medical student, and his father’s hypertension was verified from the records of the family practitioner. These subjects were compared with 12 age-matched and sex-matched normotensive controls with no family history of hypertension (– FH). All subjects were taking an unrestricted omnivore diet. It is acknowledged that despite meticulous selection misclassification of subjects in the + FH and – FH groups is possible; however, the consequences of such an event will only be to dilute any differences observed between the two groups.

On the day of the study, subjects were asked to lie supine and their blood pressure was measured by mercury sphygmomanometer and recorded. A cannula was inserted into a peripheral vein, blood was immediately drawn for plasma norepinephrine estimation, and the line was flushed with normal saline. Twenty minutes later, the blood pressure was remeasured and further blood collected for norepinephrine estimation and for leukocyte sodium transport studies. Following a further period of 20 minutes, the blood pressure was recorded and the subjects underwent a cold pressor test, based on the method of Hines and Brown. However, instead of incubating the cells in synthetic tissue culture medium, all experiments were performed using the subject’s own plasma. At 20 minutes and during the cold pressor test, 200 ml of blood was drawn into Vacutainer tubes containing lithium heparin as an anticoagulant (Becton Dickinson, Rutherford, NJ, USA). Then, 10 ml of blood was centrifuged, and the plasma was removed and stored at −70°C for later estimation of norepinephrine. Fifty milliliters of blood was transferred to universal containers, each containing 7.5 ml of Plasmagel (Uniscience, Cambridge, England), and thoroughly mixed. The containers were allowed to stand in a water bath at 37°C, which facilitated the sedimentation of erythrocytes. After 30 minutes, the supernatant was transferred to plastic 10-ml centrifuge tubes (Sarstedt, Rommelsdorf, West Germany) and centrifuged at 37°C at 300 g for 7 minutes. This formed a cell plug containing leukocytes at the bottom of the tube. The supernatant was removed, and the remaining red blood cells were destroyed by hypotonic lysis, by adding 2 ml of water and, 13 seconds later, 2 ml of 2 × Earle’s solution. This cell suspension was centrifuged at 37°C at 300 g for 5 minutes, and a pellet formed that contained leukocytes and a little red blood cell debris. The pellet was suspended in 6 ml of plasma obtained by centrifuging the remaining 140 ml of venous blood to remove the plasma and keeping it at 37°C in a water bath until required. The suspension was then labeled with 5 pCi 22Na (Amersham, Buckinghamshire, England) and incubated at 37°C for 30 minutes to reach a steady state. The cells were then centrifuged for 3 minutes at 37°C at 300 g and resuspended in 6 ml of unlabeled plasma to remove the isotope on the outside of the leukocytes. This procedure was repeated, and the cell suspension then was split into two aliquots of 3 ml each. To one of these was added 0.1 ml of 10−3 M ouabain, and samples were taken from both aliquots at regular intervals over 20 minutes during incubation at 37°C. Each pair of samples was spun at 2000 g to stop sodium efflux and precipitate the cells. The supernatant was removed, and the tube was dried with paper tissue, and residual radiactivity in the cell pellet was measured. The total sodium efflux rate constant was calculated from the slope of the linear regression curve in the absence of ouabain. Glycoside-sensitive activity was derived by subtracting the rate constant obtained in the presence of ouabain from the total.

The studies on leukocytes removed 20 minutes into the experiment were performed using plasma obtained at the same time to expose the cells to the same levels of catecholamines throughout the experiments. Similarly, cells obtained during the cold pressor test were incubated in plasma taken at the same time. Separate studies were performed on six plasma samples (from 3 + FH and 3 – FH subjects) placed in a water bath at 37°C, and an aliquot was removed at time zero and after 75 minutes for norepinephrine estimation. This time was used because the total duration of the experiment was approximately 70 minutes; therefore, it was important to ensure that plasma norepinephrine concentrations did not change throughout this period.

Norepinephrine estimations were obtained using a modification of the method of Hallman et al. Plasma samples were taken, and dihydroxybenzylamine was added to each aliquot as an internal standard. The mixture was buffered to pH 7.8 to 8.2 with Tris HCl buffer (pH 8.6), and acid-washed alumina was added. The solution was mixed for 15 minutes, the supernatant was removed, and the alumina was washed with ice-cold distilled water. After the addition of 250 μl of 0.1 M perchloric acid to extract the catecholamines, the samples were injected into a high-performance liquid chromatograph with electrochemical detector (Model BAS LC3A/LC17, Anachem, Luton, UK).

Statistical analysis was performed using Student’s unpaired t test for between-group analysis and Student’s paired t test for within-group analyses; Dunnett’s correction was used for multiple comparisons. Results are expressed as means ± SEM.
Results

The characteristics of the two groups are shown in Table 1; the groups were well matched with similar sex distribution. The −FH subjects were older (p < 0.05), but adjustment for age did not affect the results. There was no significant difference in weight or blood pressure.

Plasma Norepinephrine Concentration

Mean plasma norepinephrine concentration was unchanged when compared at time zero and at 75 minutes (2.3 ± 0.2 vs 2.4 ± 0.2 nmol/L; n = 6). At time zero there was no significant difference in plasma norepinephrine levels between −FH and +FH subjects (Table 2). At 20 minutes norepinephrine levels fell in both groups, but more in −FH than in +FH subjects. The difference between the two groups was not significant after Dunnett’s correction (see Table 2). During the cold pressor test both groups showed a significant rise in plasma norepinephrine concentration; the levels in +FH subjects were higher than those in −FH subjects, although this difference did not attain statistical significance (see Table 2).

Blood Pressure

There was no significant difference between the blood pressures of the two groups at time zero (Table 3). Both groups showed falls in systolic blood pressure at 20 minutes, but there was no significant change at 40 minutes (see Table 3). The diastolic pressures rose slightly in both groups at 20 minutes, but they rose no further between 20 and 40 minutes. Both groups showed a highly significant rise in systolic and diastolic pressure after 60 seconds of the cold pressor test (p<0.001; see Table 3).

Leukocyte Sodium Efflux Rate Constant

There was no significant difference 20 minutes before the cold pressor test in mean total, ouabain-resistant or ouabain-sensitive efflux rate constants between −FH controls and +FH subjects (p>0.1; Table 4). The mean leukocyte sodium efflux rate constants in the −FH group were unchanged during the cold pressor test compared with the values obtained at 20 minutes (see Table 4). However, when the change in norepinephrine was plotted against change in total efflux rate constant, a highly significant negative correlation was noted (r = −0.82, p<0.01, n = 12; Figure 1). A different pattern emerged in the +FH group: there was a significant rise in ouabain-resistant efflux rate constant (p<0.01; see Table 4) and a nonsignificant fall in ouabain-sensitive efflux rate constant with the cold pressure.

<table>
<thead>
<tr>
<th>Table 1. Physical Characteristics of Study Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>No. of subjects</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Age (yr)</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Most values are means ± SEM. −FH = no family history of hypertension; +FH = family history of hypertension (1 or both parents).

<table>
<thead>
<tr>
<th>Table 2. Plasma Norepinephrine Concentrations in Subjects With and Without a Family History of Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>−FH</td>
</tr>
<tr>
<td>+FH</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Between-group differences were not significant. CPT = cold pressor test. See Table 1 for key to other abbreviations.

<table>
<thead>
<tr>
<th>Table 3. Blood Pressures in Subjects With and Without a Family History of Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>−FH</td>
</tr>
<tr>
<td>Systolic</td>
</tr>
<tr>
<td>Diastolic</td>
</tr>
<tr>
<td>+FH</td>
</tr>
<tr>
<td>Systolic</td>
</tr>
<tr>
<td>Diastolic</td>
</tr>
</tbody>
</table>

Values are means ± SEM. CPT = cold pressor test. See Table 1 for key to other abbreviations.

Table 4. Mean Leukocyte Sodium Efflux Rate Constants in Subjects With and Without a Family History of Hypertension at 20 Minutes and During Cold Pressor Test

<table>
<thead>
<tr>
<th>Time</th>
<th>−FH Constants (hr⁻¹)</th>
<th>+FH Constants (hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Ouabain-resistant</td>
</tr>
<tr>
<td>20 min</td>
<td>3.1±0.13</td>
<td>0.9±0.13</td>
</tr>
<tr>
<td>During CPT</td>
<td>3.0±0.12</td>
<td>0.9±0.1</td>
</tr>
</tbody>
</table>

Values are means ± SEM. CPT = cold pressor test. See Table 1 for key to other abbreviations.

*p < 0.01, compared with value at 20 minutes.

†p < 0.002, compared with value in −FH subjects.
pressor test. When compared with the values observed in FH subjects, there was a significant increase in ouabain-resistant efflux rate constant ($p<0.002$; see Table 4). Ouabain-sensitive efflux rate constant was depressed but not significantly different from FH control values ($p=0.073$). There was a significant positive correlation between change in norepinephrine and change in total efflux rate constant ($r=0.64$, $p<0.05$, $n=10$; Figure 2). The slopes of the regression lines of the two correlations were significantly different from each other ($t=16.582$, $p<0.0001$).

**Discussion**

These results demonstrate that a stressful stimulus such as exposure to cold can influence leukocyte sodium efflux rate constant, producing a different pattern of response in +FH subjects compared with FH controls. Thus, while no changes in mean values were observed in FH subjects, there was a highly significant negative correlation between plasma norepinephrine changes and changes in total efflux rate constant. In +FH subjects, ouabain-resistant efflux rate constants rose significantly and a positive correlation between plasma norepinephrine changes and changes in total efflux rate constant was observed.

The cold pressor test has been used to produce transient blood pressure elevation for 50 years. The pressure effect is associated with rises in both plasma norepinephrine and epinephrine and probably a variety of other hormones. One study found that an exaggerated pressor response can delineate children at risk of becoming hypertensive in later life. Moreover, Falkner et al. have demonstrated an enhanced cold pressor response in the first-degree relatives of essential hypertensive patients. This was not the case in our study, however. This discrepancy is probably attributable to the recording in previous studies of the highest reading obtained after immersion as the ceiling blood pressure. This reading often occurs before 60 seconds has elapsed, which was the time in our protocol when the blood pressure was measured. Nevertheless, judging by the elevation achieved, the experiment successfully stressed the subjects.

The results obtained suggest that stress can influence leukocyte sodium transport but is dependent on family pedigree. No differences were observed in sodium transport characteristics between +FH and FH subjects after 20 minutes of supine rest. During the cold pressor test, however, ouabain-resistant efflux rate constant rose significantly in +FH subjects, whereas ouabain-sensitive rate constant was unaltered. Ouabain-resistant flux is complex and includes Na⁺-K⁺ countertransport, Na⁺-K⁺ cotransport, and passive permeability in membranes. It is therefore not possible to ascertain precisely which mechanism was stimulated in these experiments. Examination of these transport systems in hypertensive patients and their normotensive offspring has produced reports of raised Li⁺-Na⁺ countertransport in erythrocytes from these subjects, and this pathway has been shown to be reduced after a 12-week exercise program, a maneuver that may reduce plasma catecholamine levels. Indeed, the mechanism of stress-induced alterations in membrane handling of sodium is unclear. The plasma concentrations of a number of hormones are raised by stress, and the levels of norepinephrine were measured as an index of the degree of stress induced by the cold pressor test. Thus, although correlations between total sodium efflux rate constant and norepinephrine were observed in both groups, this is unlikely to be the only humoral factor at work. Because a correlation has been demonstrated it would be unsafe to assume a causal relationship.

The differences in response to the cold pressor test in the two groups studied deserve comment. In a previous study we demonstrated similar changes in the ouabain-resistant leukocyte sodium efflux rate con-
stant in + FH subjects on extremes of salt intake, with no corresponding changes in − FH controls. Again, the mechanism mediating the changes may be stress-related, but the identical pattern of response in + FH subjects in the two studies makes it attractive to suggest that both experiments produced a further manifestation of a global difference in the plasma membrane inherited by persons at risk of having raised blood pressure in later life. However, the possibility of hormones other than norepinephrine being differentially secreted in + FH and − FH subjects in stress-related circumstances cannot be ignored, and the presence of a genetically altered plasma membrane must remain speculative at this time. Nevertheless, the leukocyte sodium transport characteristics of + FH and − FH subjects were dissimilar during the cold pressor test. The exact nature of the enhanced ouabain-resistant pathway potentiated during stress in + FH subjects remains to be determined, and while attractive, the possibility of a structural membrane abnormality in + FH subjects underlying their different response requires further investigation before being confirmed.

Acknowledgment

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References

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