Effect of Vitamin C in Modulating the Hypothermic Influence on Nerve Conduction


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Abstract: The study was carried out to evaluate the effect of different degrees of hypothermia on nerve conduction and the possible beneficial effect of vitamin C in the amelioration of the impairment in nerve conduction due to hypothermia. Sixty male Wistar rats, 225–250 g, were randomly divided into two equal groups of untreated controls and vitamin C treated experimental groups. Sciatic nerve conduction and nerve temperature ($T_n$) were recorded at different degrees of hypothermia by step-wise lowering of rectal temperature ($T_r$) from 38 to 20°C. A regression analysis showed a positive linear relationship of $T_r$ with nerve conduction velocity (NCV) and with $T_n$ in both groups ($p<0.001$). There was also a positive linear relationship between $T_n$ and NCV in both groups: control, $p<0.001$; experimental, $p<0.05$. Comparisons between the regression equations of $T_r$ with NCV, $T_r$ with $T_n$, and $T_n$ with NCV showed significant differences between the two groups ($p<0.001$). There was no significant relationship between $T_r$ and the amplitude of the action potential. Vitamin C may have a protective effect against the impairment of NCV due to hypothermia. [Japanese Journal of Physiology, 46, 397–402, 1996]

Key words: hypothermia, nerve conduction, vitamin C.

Exposure to an extreme cold environment that could lead to hypothermia is a serious medical emergency. Such a situation may arise when induced to a polar environment without proper protection such as accidents in flight or even when mountaineering at extreme altitudes. The problem is of direct relevance to defence personnel who operate under acute cold conditions. Impaired nerve conduction is one of the complications resulting from hypothermia. Studies on nerve conduction carried out so far have mainly evaluated the effect of skin temperature on nerve conduction [1, 2]. Hence it was thought worthwhile to study the relationship between core temperature and nerve conduction. In order to evaluate the effects of prior supplementation with a natural or synthetic compound against the impairment of nerve conduction, the effect of vitamin C supplementation was studied. Our interest in vitamin C was kindled by previous reports from this laboratory showing its beneficial effects on cold injuries [3]. Recent studies have also highlighted the role of vitamin C in the regulation of cellular and enzymatic functions [4].

This study aimed to (a) establish the relationship of rectal temperature ($T_r$) with nerve conduction and nerve temperature ($T_n$) as well as the relationship between $T_n$ and nerve conduction. The relationship was studied over a much wider range of temperature than reported before (b) to study the possible beneficial effect of vitamin C on the amelioration of the impairment in nerve conduction due to hypothermia.

METHODS

Nerve conduction was studied in 60 male Wistar rats weighing between 225 and 250 g. The study was approved by the Ethics Committee of the Institute and National Institute of Health (NIH) principles of laboratory care (NIH publication No. 85-23, revised 1985) were followed. The experiments were carried out double blind. The rats were randomly assigned into two
equal groups: the first group consisted of experimental rats administered vitamin C orally and the second group being the control rats was administered 0.5 ml water.

Experiments were carried out in an air-conditioned laboratory at room temperature 25 ± 1°C. Ascorbic acid, 50 mg (0.5 ml of 100 mg/ml solution) was administered once just before anesthesia and the experiment was carried out 1-2 h after the administration of vitamin C. A plastic syringe with a 16 mm gauge needle blunted at the top surface, which had a plastic cannula fixed to it, was used for administration. The rats were anesthetized using urethane intraperitoneally using a dosage of 1.5 g/kg bw. The right sciatic nerve was exposed from the vertebral origin to its insertion in the gastrocnemius muscle. A paraffin pool was made. Rectal and nerve temperatures (T_r) were monitored using a telethermometer (YSI model 46 TUC). The rectal probe was dipped in liquid paraffin, inserted into the rectum and secured by adhesive plaster to the tail. The nerve temperature was recorded at different points by placing the sciatic nerve over the probe in order to ensure uniformity of temperature. A typical recording of the nerve temperature at different points along the nerve is shown in Table 1. Bipolar Ag–AgCl electrodes were used for stimulation and recording. The electrodes were fixed in place with a clamp. The stimulating electrodes were placed at the vertebral end, 0.5–1.0 cm lateral to the point of origin and recording electrodes at the muscle end (gastrocnemius) close to the muscle belly. A thread was tied to the point of insertion. The nerve was sectioned at the muscle end and its branches were cut in order to avoid interferring potentials. Care was taken to retain the blood supply.

The preparation was stimulated using square wave pulses of 0.1 ms duration at a supra-threshold voltage (4–5 V) using a stimulator (Grass S-88, Quincy, MA) connected to a Stimulus Isolation Unit (Grass, SIU 5A). The responses were amplified using a physiological amplifier (Model HG A-200 A, Nicolet, Madison, WI) and displayed on an oscilloscope (Model 5110, Tektronix, Beaverton, OR) using the Clinical Averaging Programme of a Nicolet MED-80 data processor and printed on a teletype printer (Model 43).

The recordings were obtained at T_r between 38 and 20°C, which was lowered step-wise by placing the animal on a rubber bag containing crushed ice. Five recordings were averaged over every 2°C fall in T_r and a single response was obtained. Recordings were taken only after the rectal and nerve temperatures remained steady for at least 30 s. The distance between the midpoint of the stimulating and recording electrodes was measured to the nearest mm using a divider. The latent period (ms) was measured as the distance between the stimulus artifact and the first positive peak of the response. The amplitude (mV) of the response was measured peak to peak.

**Statistical procedure.** A linear regression analysis was performed to establish the relationship of NCV, amplitude of the compound action potential and T_r with T_r. The relationship of NCV with T_r was also established. The significance of regression equations between the control and experimental groups was tested by using t-test.

**RESULTS**

The linear regression analysis showed that NCV was positively correlated with T_r in the control (p<0.001) and experimental (p<0.001) groups. Figures 1 and 2 show the relationship between T_r and NCV in the two groups. Figure 3 shows tracings of the action potentials recorded from a control and an experimental rat. Step-wise lowering of rectal temperature led to an increase in the latent period and hence, a reduced con-

<table>
<thead>
<tr>
<th>T_r (°C)</th>
<th>Distance from stimulating electrode (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>38</td>
<td>32.6</td>
</tr>
<tr>
<td>30</td>
<td>24.1</td>
</tr>
<tr>
<td>22</td>
<td>21.8</td>
</tr>
</tbody>
</table>

Fig. 1. Relationship between nerve conduction velocity and rectal temperature in the control group (n=30).
Vitamin C and Hypothermia

Conduction velocity in the control group. Vitamin C treated rats showed faster conduction velocities at lower rectal temperatures as compared to the control group. There was no significant linear correlation between the \( T_r \) and amplitude of the response in either group. Table 2 shows the mean amplitude at different levels of \( T_r \). There was no significant difference between the regression equations of \( T_r \) with amplitude in the two groups. A regression analysis of \( T_r \) with \( T_n \) showed a positive correlation in the control (\( p<0.001 \)) and experimental (\( p<0.001 \)) groups. Figures 4 and 5 show the relationship between \( T_r \) and \( T_n \) in the two groups. As shown in Figs. 6 and 7, there was also a significant correlation between \( T_n \) and NCV in the control (\( p<0.001 \)) and experimental (\( p<0.05 \)) groups.

The relationship between \( T_r \) and NCV was expressed by the equations:

**Control group**

\[
NCV = -41.27 + 2.9T_r
\]

\( r=0.84, p<0.001 \)

**Experimental group**

\[
NCV = 13.45 + 1.39T_r
\]

\( r=0.38, p<0.001 \)

The relationship between \( T_r \) and \( T_n \) was expressed by the equations:

**Control group**

\[
T_n = 16.00 + 0.3T_r
\]

\( r=0.95, p<0.001 \)

**Experimental group**

\[
T_n = 14.67 + 0.4T_r
\]

\( r=0.89, p<0.001 \)

The relationship between \( T_n \) and NCV was expressed by the equations:

![Graph showing the relationship between nerve conduction velocity and rectal temperature in the experimental (vitamin C treated) group (n=30).](image)

**Fig. 2.** Relationship between nerve conduction velocity and rectal temperature in the experimental (vitamin C treated) group (n=30).

**Table 2.** Amplitude (mV) of the compound action potential in control (n=30) and experimental (vitamin C treated) groups (n=30) at different rectal temperatures.

<table>
<thead>
<tr>
<th>( T_r ) (°C)</th>
<th>38</th>
<th>36</th>
<th>34</th>
<th>32</th>
<th>30</th>
<th>28</th>
<th>26</th>
<th>24</th>
<th>22</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Mean</td>
<td>1.59</td>
<td>2.35</td>
<td>1.15</td>
<td>1.03</td>
<td>1.85</td>
<td>1.69</td>
<td>2.40</td>
<td>1.85</td>
<td>2.02</td>
<td>2.49</td>
</tr>
<tr>
<td>SD</td>
<td>1.18</td>
<td>1.70</td>
<td>0.31</td>
<td>0.66</td>
<td>1.06</td>
<td>0.95</td>
<td>0.14</td>
<td>1.60</td>
<td>1.13</td>
<td>0.11</td>
</tr>
<tr>
<td>Experimental Mean</td>
<td>1.07</td>
<td>0.91</td>
<td>1.21</td>
<td>1.33</td>
<td>1.60</td>
<td>1.29</td>
<td>1.57</td>
<td>1.50</td>
<td>1.63</td>
<td>1.11</td>
</tr>
<tr>
<td>SD</td>
<td>1.11</td>
<td>0.56</td>
<td>0.65</td>
<td>0.29</td>
<td>0.99</td>
<td>0.43</td>
<td>0.92</td>
<td>0.77</td>
<td>1.57</td>
<td>0.29</td>
</tr>
</tbody>
</table>


399
Control group

\[ \text{NCV} = -170.5 + 8.6T_n \]
\[ (r=0.66, p<0.001) \]

Experimental group

\[ \text{NCV} = -24.9 + 3.0T_n \]
\[ (r=0.13, p<0.05) \]

On comparison of the regression equations of the control and experimental groups of \( T_r \) with NCV, a significant difference was found in the intercept \((p<0.001)\) and the slope \((p<0.001)\). Comparisons of the regression equations of \( T_r \) with \( T_n \) in the two groups showed a significant difference in the intercept \((p<0.001)\) and the slope \((p<0.001)\). There was also a significant difference in the intercept \((p<0.001)\) and slope \((p<0.001)\) in the regression equations of the control and experimental groups of \( T_n \) with NCV.

Values of NCV showed a change of 3 m/s/°C in the \( T_r \) between 38 and 20°C in the control group and 1.5 m/s/°C in the experimental group (i.e., 4%/°C change and 2%/°C change in \( T_r \) in the control and experimental groups respectively). The fall in NCV between 38 and 20°C was 73.7 and 36.9% in the control and experimental groups, respectively. At a \( T_r \) of 26°C, NCV was reduced to about half of the normal value (52.9%) in the control group and about two-thirds of the value (32%) in the experimental group. This \( T_r \) corresponded with a \( T_n \) of 24.2 and 24.6°C in the control and experimental groups, respectively. At a \( T_n \) of 21°C (\( T_r = 20°C \)), the NCV of the control group was about 10 m/s, whereas it was 40 m/s in the experimental group.

**DISCUSSION**

A positive correlation of \( T_r \) with NCV was found, indicating that NCV is reduced by hypothermia. The differences in the regression equations of the control and experimental groups indicate that the administration of vitamin C alters the relationship in such a way that NCV falls less as \( T_r \) is lowered. There were also positive linear relationships between \( T_r \) and \( T_n \) and NCV. A faster NCV at lower rectal temperatures may be due to the reduced slope of the \( T_n \)–NCV relationship.

Conduction velocity is normally determined by the structural characteristics of the nerve (i.e., its fiber diameter, myelin thickness, and nodal properties) [5].
The effect of temperature may be on the sodium channel kinetics [6]. At lower temperatures, the channels may be open for a longer period as it may take longer for the outward current at the next nodal region to depolarize the nodal membrane at the threshold, leading to slower impulse conduction. As the rectal temperature is lowered, the activity of Na-K ATPase is reduced. Secondly, there is generation of free radicals [7]. It is well known that vitamin C is a free radical scavenger and protects the membrane from free radical damage. It may be speculated that vitamin C may also help in maintaining the function of the Na-K pump, therefore, maintaining normal membrane and action potentials.

The percentage of change in NCV/°C in response to T_r in this study is in close agreement with the values reported regarding mouse sciatic nerve [8]. The reduction of 3 m/s/°C in the reduction of NCV/°C in response to T_r is also comparable with the values reported (2.2-2.6 m/s) in human subjects per °C fall in skin temperature [9-11]. No change in the amplitude was found with change in temperature. This finding is in agreement with recent studies on human subjects [1, 12]. Some researchers [13, 14] found increased amplitudes with lower temperatures, whereas some earlier studies reported a reverse trend [15, 16]. Amplitude may be determined by the ratio of the counteracting effects of cooling (which may increase the amplitude) and by the temporal dispersion of the impulses due to cooling (which may decrease the amplitude) [12]. This may explain the lack of any trend in amplitude of the potential compound action as the temperature is lowered.

This study had an additional advantage that both rectal and nerve temperatures were simultaneously recorded. Not surprisingly, the correlation between T_r and T_n was highly significant (r=0.95, p<0.001). Recordings at different points along the nerve length did not reveal significant differences in T_n; it is assumed that lowering the core temperature would result in lower nerve temperature. Under the experimental conditions, T_n would also be influenced by the temperature and air currents of the surrounding microenvironment. Since we maintained the microenvironment constant, it is believed that T_n was not affected by this parameter.

Recent reports have highlighted the beneficial effect of the intake of mega quantities of ascorbic acid for a wide range of stresses. Stress is accompanied by depleted levels in particular tissues (e.g., leukocytes) [17, 18]. The physiological synthesis of vitamin C in rats also increases in various stress situations (e.g., at low ambient temperatures). Previous studies by Dugal et al. reported the beneficial effects of vitamin C in cold acclimatization accompanied by increased concentrations in specific tissues [19]. Hence, the optimal levels may depend upon the body state. In this study, the level of endogenous synthesis in the control group is apparently not sufficient to meet the hypothermic stress. On the other hand, the dosage of vitamin C administered in the experimental group may suppress the endogenous synthesis by a feedback control mechanism which may react by decreasing production from the liver [20].

In human subjects, hypothermia and severe cold exposure reduce mental performance [21, 22], bring about changes in brain electrical activity [23], decrease muscular performance and alter EMG activity.
In a study on conscious human subjects [23] at a core temperature of 33.5°C, a shift of EEG waves towards increased theta and beta activity and decreased alpha activity was reported. The visual evoked potential latencies were found to be slightly increased. Cooling may influence the peripheral conduction mechanism and CNS function. Peripheral conduction will be hampered as neuromuscular functions may be impaired since nerve membrane is less excitable and conduction velocity is reduced. The ability to process information may become impaired. The central nervous functions may be impaired as a result of cooled blood perfusing the CNS [23]. The impairment of conduction due to cooling may be ameliorated by vitamin C. Thus, it may prove to be a useful prophylactic agent for supplementation in field situations before anticipated cold exposure.

REFERENCES

18. Stone I: The Healing Factor, Vitamin C against Disease, Grosset and Dunlap, New York, 1972