Catecholamine levels in divers subjected to stresses of immersion and hyperbaric exposure

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Manalaysay AR, Langworthy HC, Layton RP. Catecholamine levels in divers subjected to stresses of immersion and hyperbaric exposure. Undersea Biomed Res 1983; 10(2):95–106.—The study was undertaken to determine the changes in plasma catecholamine levels in response to the combined stresses of cool water immersion and hyperbaric exposure. Plasma catecholamines were measured in seven thermally unprotected trained male U.S. Navy divers immersed in water at 25°C and 35°C at 1 ATA and 4 ATA. All measurements were made prior to any decompression procedures. Plasma norepinephrine (NE) levels were higher during cool immersions at both 1 ATA and 4 ATA. Hyperbaric exposure during warm immersion was associated with a small but significant increase in plasma NE levels. Hyperbaric exposure during cool immersion was associated with an increase in plasma NE levels, but this increase was not statistically significant. Hyperbaric exposure in both the cool and warm immersions was associated with a moderate degree of hypoventilation and carbon dioxide retention. Plasma NE levels reflect the overall stress imposed on an individual. This study indicates that plasma NE levels may be too variable to be useful as indicators of specific stress.

catecholamines
stress
pressure

temperature
hypercapnia
alveolar ventilation

Catecholamines are a class of compounds that have an amine group attached to a catechol nucleus. Two of the more common members of this class are norepinephrine (NE) and epinephrine (E). Unless otherwise specified, reference to NE and E will be assumed when catecholamines are mentioned in this text. Catecholamines are readily found in the tissues and circulating fluids of mammals. They are believed to be synthesized in the brain, chromaffin cells, and sympathetic nerve endings (1–3). The catecholamines are known to have multiple physiological and metabolic effects, and are known to be important in the modulation and control of the cardiovascular, respiratory, and thermoregulatory systems (4). Circulating free catecholamines are taken up by an organ in direct proportion to that organ’s fractional share of the cardiac output (5). For example, the heart gets a large proportion of the cardiac output relative to its tissue weight and has a large number of sympathetic nerve endings; it thus takes up a large amount of the circulating catecholamines (5–8).

Catecholamine levels in the plasma are affected by the overall stress to which the organism is being exposed (9–13). Within broad limits, the relation of the catecholamine levels to the
type of stress is relatively nonspecific. However, varying the type of stress or the degree of stress will result in different changes in the circulating catecholamines. For example, it has been shown that exposure of subjects to moderate cold will cause NE levels in the plasma to rise. Severe cold exposure will cause the plasma levels of both NE and E to rise (14).

Diving is a risky undertaking even when performed under ideal conditions; the risks are even greater under the usual working dive conditions. Divers need efficient cardiovascular, respiratory, and thermoregulatory systems to cope with the underwater environment. As indicated above, catecholamines are important in the modulation and control of these systems. In this study we sought to examine the effect of hyperbaric exposure on plasma catecholamine levels in resting divers during immersion in cool and warm water.

MATERIALS AND METHODS

Seven male U.S. Navy divers were used throughout the study. All procedures were fully explained to the subjects, and informed consent was obtained from each diver prior to the beginning of the study. On the evening prior to the dive, the diver had a regular meal and avoided stimulants and any unusual stress. The diver reported on the day of the dive in a fasting state and was instrumented to allow continuous monitoring of the electrocardiogram as described elsewhere (15). A plastic indwelling catheter was inserted into a forearm vein to allow venous blood sampling while the subject was immersed.

The site of the venipuncture was cleansed with a povidone-iodine scrub solution and allowed to dry. A standard indwelling plastic catheter was inserted and anchored with plastic tape. Seepage of water was prevented by applying a layer of plastic tape to the skin surrounding the puncture site, applying a collodion solution liberally around the puncture site, and finally placing another layer of plastic tape over the exposed part of the catheter hub assembly. This last layer of tape was carefully laid down to ensure good contact with the first layer of tape. A YSI 700-series linear thermistor (Yellow Springs Instruments, Yellow Springs, OH) was inserted 10 cm into the rectum to allow continuous rectal temperature monitoring.

Ten Thermonetics heat flow sensors (Thermonetics Corp., San Diego, CA) were affixed to various parts of the body according to the method of Layton (16, 17). This basically involves attaching heat flow sensors to the chest, upper back, abdomen, lower back, upper arm, forearm, the back of the hand, the thigh (two sensors), and the calf. The sensors were attached with an adhesive used in colostomy bag applications. The measured heat flow from each of these sites was combined and used to estimate regional as well as whole-body heat loss. This method of calorimetry gives results that compare very favorably with the tube suit calorimeter and the bath calorimeter.

A Plexiglas tank was placed inside a pressure chamber and was used for all immersions. The temperature of the water in the immersion tank was kept constant, ± 0.1°C using an external water bath. The external water bath was mounted outside the chamber containing the immersion tank. Cooling fluid from the bath was pumped into a closed system of copper tubing coils that were located inside the immersion tank. Passage of the copper tubes into and out of the pressure chamber was accomplished through appropriate penetrators. A thermistor mounted inside the immersion tank provided input to the water bath temperature controller. During the studies involving hyperbaric exposures, the compressions and decompressions were performed in accordance with standard procedures as outlined in the *U.S. Navy Diving Manual* (18).

The breathing gas used throughout the study was compressed air; this was delivered to the diver via a double-hose demand regulator, modified to allow collection of the expired gas. The second stage of the regulator was located on the back of the diver at the level of the sternal
notch. Exhaled gas was channeled to a 100-liter Douglas bag. The volume of the exhaled gas in the Douglas bag was measured with a Tissot spirometer (Warren E. Collins, Inc., Braintree, MA) after a 500-ml aliquot had been removed for gas analysis. An Applied Electrochemistry S-3A oxygen analyzer (Applied Electrochemistry, Inc., Sunnydale, CA) and a Beckman Instruments (Fullerton, CA) LB-2 carbon dioxide analyzer were used for the gas analysis. The gas analyzers were calibrated prior to each analysis using Matheson Gold Calibration Standard gases (Matheson, Secaucus, NJ), which were gravimetrically prepared and certified accurate to a stated concentration, ± 0.02%.

During a typical experiment the diver wore swim trunks and was instrumented as described above. The diver then entered the water and sat totally submerged at rest on a plastic chair in the tank. Three 10-ml blood samples were collected at approximately 14-min intervals during the experiment. Six exhaled gas samples were collected at approximately 7-min intervals during the experiment. Each sample was collected over a 2-min period. The electrocardiogram, rectal temperature, and heat flow sensors were monitored continuously. Data from these sources were sampled intermittently on a prearranged schedule. Each diver was studied under four conditions: 1) immersion in 35°C water at 1 ATA; 2) immersion in 25°C water at 1 ATA; 3) immersion in 35°C water at 4 ATA; 4) immersion in 25°C water at 4 ATA. The experimental conditions for a given day were varied in a random fashion. In the studies performed at 4 ATA the compression was carried out before the diver entered the immersion tank.

All measurements and sample collections were carried out prior to any decompression procedures. A compressed air centrifuge was used for the plasma separations. Catecholamine analysis was performed radioenzymatically, using the CAT-A-KIT produced by Upjohn Diagnostics (Kalamazoo, MI). This method is a modification of the simultaneous single-isotope technique described by Peuler and Johnson (19). The typical accuracy for NE measurements by this method is ± 10%.

Oxygen consumption ($\dot{V}_O_2$), carbon dioxide production ($\dot{V}_C_0_2$), and minute ventilation ($\dot{V}_E$) were measured at various points in the experiment. Appropriate calculations were made to express these variables both at btps and stpd (20). $\dot{V}_C_0_2$ is expressed in liters/min stpd (21). Respiratory dead space volume (VDS) was estimated by using the weight of the subject in pounds (22). Alveolar ventilation was estimated as follows:

$$\dot{V}_A = \dot{V}_E - (f \times VDS) \quad (1)$$

where $\dot{V}_A$ = alveolar ventilation (liters/min btps), $\dot{V}_E$ = minute ventilation (liters/min btps), $f$ = respiratory rate (breaths/min), $VDS$ = respiratory dead space volume (liters btps). The alveolar partial pressure of carbon dioxide ($P_{ACO_2}$) was calculated using the equation

$$P_{ACO_2} = 863 \times (\dot{V}_C_0_2 \dot{V}_A) \quad (2)$$

where $\dot{V}_C_0_2$ and $\dot{V}_A$ are as defined above (21) and $P_{ACO_2}$ is in mmHg. Analysis of variance techniques were applied to the collected data.

The statistical treatment of the data was based on a model that assumes that the NE levels in a person completely immersed in water will be proportional to the duration of the immersion. In this model the effect of pressure on the NE levels, if any, would be to introduce a constant difference in the NE levels at any given time. Thus, if the data were to be plotted with the NE levels on the Y axis and time on the X axis, data obtained at one pressure level would fall along a line with a given intercept on the Y axis. Data obtained at another pressure would fall along a line with the same slope but with a different Y-axis intercept. The magnitude of the Y-axis intercept offset would be a function of the magnitude of the pressure differential. No account
was made for the use of repeated measurements on the same subject, since inter- and intra-subject variability appeared comparable.

The NE levels at a given temperature (warm or cool) were plotted against time of immersion. The null hypothesis stated that the pressure effect was zero; thus all the NE levels at a given temperature fell on a single line. The test hypothesis stated that pressure did have an effect on the NE levels and that, at a given temperature, the NE values at 4 ATA fell on one line, and the NE values at 1 ATA fell on another line. The $F$ ratios were calculated by the method of the sum of squares error terms to determine if there was a significant difference in intercept between the two lines.

RESULTS

The plasma NE levels during cool immersion were found to be significantly higher than the plasma NE levels during warm immersion both at 1 ATA and 4 ATA (Fig. 1, Table 1). The immersion studies at 4 ATA were associated with slightly higher plasma NE levels than those seen at 1 ATA, and this was true for both cool and warm immersions. The plasma E levels during cool immersion were higher than those seen during warm immersion (Fig. 2). There was a minimal difference in the plasma E levels during 4 ATA vs. 1 ATA immersions.

![Graph showing NE levels vs. time for 1 and 4 ATA in cool and warm conditions]

**Fig. 1.** Plasma norepinephrine (pg/ml) vs. time of immersion (min). Each point represents a mean value obtained from the seven divers at the indicated immersion time for each of the four conditions in the study. Lines are drawn through the points derived from each of the four experimental conditions. NE, norepinephrine.
### TABLE 1
**Mean Plasma Norepinephrine**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>1 ATA Warm</th>
<th>4 ATA Warm</th>
<th>1 ATA Cool</th>
<th>4 ATA Cool</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pg/ml</td>
<td>SD</td>
<td>pg/ml</td>
<td>SD</td>
</tr>
<tr>
<td>14</td>
<td>166</td>
<td>61</td>
<td>555</td>
<td>286</td>
</tr>
<tr>
<td>29</td>
<td>175</td>
<td>68</td>
<td>692</td>
<td>307</td>
</tr>
<tr>
<td>44</td>
<td>197</td>
<td>80</td>
<td>812</td>
<td>262</td>
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<tr>
<td></td>
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<td>237</td>
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<td>125</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
<td>222</td>
</tr>
</tbody>
</table>

$n = 7.$

**Fig. 2.** Plasma epinephrine (pg/ml) vs. time of immersion (min). Each point represents a mean value obtained from the seven divers at the indicated immersion time for each of the four conditions in the study. Lines are drawn through the points derived from each of the four experimental conditions. E, epinephrine.

Figure 1 shows that there is a highly significant increase ($P < 0.001$) in the plasma NE levels in cool vs. warm immersion. The same figure also indicates that there is a difference in the plasma NE levels at 1 ATA vs. 4 ATA in both warm and cool immersions. Statistical analysis of the data shows that this difference is small but is significant to the 0.01 level in the warm immersions. When NE is plotted vs. time for the warm immersions, a regression plot through the 1-ATA points crosses the $Y$ axis at 115 pg/ml (Fig. 3). A similar regression plot through the 4-ATA points intersects the $Y$ axis at 186 pg/ml. This is a difference of $70 \pm 25$ pg/ml.
Fig. 3. Plasma norepinephrine (pg/ml) vs. time of immersion (min). Each point represents a mean value obtained from the seven divers at the indicated immersion time for each of the four conditions of the study. The lines are linear regressions through the data points at either 1 ATA or 4 ATA. NE, norepinephrine.

The data from the cool immersion studies show that there appears to be a difference in the NE levels at 1 ATA vs. 4 ATA. A regression plot through the 1-ATA points crosses the Y axis at 371 pg/ml; a similar regression plot through the 4-ATA points intersects the Y axis at 515 pg/ml. This is a difference of 143 ± 91 pg/ml. Statistical analysis showed that the two line plots were not sufficiently different to allow us to reject the null hypothesis for the cool immersion data. The null hypothesis stated that the effect of pressure on catecholamine levels was zero.

Examination of the raw data shows that the NE levels in the cool immersions have a greater variability than the NE levels in the warm immersions (Table 2). For example, when a line is fitted to cool immersion data points, the residual standard deviation after regression of the data points along this line is 300 pg/ml. When the warm immersion data are treated similarly, the residual standard deviation after regression is 90 pg/ml.

A plot of $\bar{V}_A$ vs. $\bar{V}_{CO_2}$ (with warm and cool immersion data combined) shows that alveolar ventilation is greater at 1 ATA than at 4 ATA for any given level of CO$_2$ production (Fig. 4). Likewise, plotting $P_{ACO_2}$ vs. $\bar{V}_{CO_2}$ shows that $P_{ACO_2}$ is consistently higher at 4 ATA than at 1 ATA for any given $\bar{V}_{CO_2}$ level (Fig. 5).

The heat flux measurements showed that 9 of the 10 sites monitored exhibited a greater rate of heat loss in the cool vs. the warm immersions as expected (Table 3). However, the heat flux measured on the dorsum of the hand was essentially identical in warm and cool immersions. The paired t test applied to the heat flux data showed that the regional heat losses at 1 ATA and 4 ATA were not significantly different.
### TABLE 2

**PLASMA NOREPINEPHRINE**

<table>
<thead>
<tr>
<th>Pressure</th>
<th>Water Temp.</th>
<th>Sample No.</th>
<th>Subject No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1 ATA</td>
<td>Cool</td>
<td>1</td>
<td>272</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>326</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>435</td>
</tr>
<tr>
<td>4 ATA</td>
<td>Cool</td>
<td>1</td>
<td>354</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>633</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>741</td>
</tr>
<tr>
<td>1 ATA</td>
<td>Warm</td>
<td>1</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>158</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>155</td>
</tr>
<tr>
<td>4 ATA</td>
<td>Warm</td>
<td>1</td>
<td>266</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>207</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>301</td>
</tr>
</tbody>
</table>

*Values in pg/ml.

**Fig. 4.** $\dot{V}_A$ (liters/min BTPS) vs. $\dot{V}_{CO_2}$ (liters/min STPD) at 1 and 4 ATA. Each point represents a mean value obtained from the seven divers at the indicated pressure levels, in both cool and warm immersions. There were 6 gas analysis points in each of the four experimental conditions. The lines represent a linear regression through the 12 gas analysis data points obtained at either 1 or 4 ATA.
Fig. 5. $P_{ACO_2}$ in mmHg vs. $\dot{V}CO_2$ (liters/min STPD) at 1 and 4 ATA. Each point represents a mean value obtained from the seven divers at the indicated pressure levels, in both cool and warm immersions. There were 6 gas analysis points in each of the four experimental conditions. The lines represent a linear regression through the 12 gas analysis data points obtained at either 1 or 4 ATA.

DISCUSSION

The increase in plasma NE levels in cool vs. warm immersions is not a new finding and has been reported before (9, 10, 14, 23). Examination of the cool data shows what appears to be a large difference in the plasma NE levels at 1 ATA vs. 4 ATA (Fig. 1). However, analysis of the data has showed that this difference is not statistically significant. This lack of significance could be due to the large variation in the measured NE levels in the cool immersions (Table 1). The radioenzymatic method used in this study (19) is very sensitive but not very reproducible. For example, serial assays performed on control plasma in our laboratory yield results with a variability in the range of ± 10%. This variability is in accord with reports from other investigators and the quality control laboratory of Upjohn Diagnostics (24).

Plasma catecholamine levels are dynamic and respond to many stimuli. Cool immersion is a potent stimulus to secretion of NE, and the individual response to this stimulus is variable. This individual variability will add to the variability inherent in the assay method, resulting in a greater overall variability in cool immersions when compared with the warm immersions. Warm immersion results in suppression of NE levels (23, 25); plasma NE measurements under these conditions have typically shown less variability in our experience.

There is reason to think that the differences in plasma NE at 1 ATA vs. 4 ATA may be even more substantial. Hesse and co-workers (26) studied the circulating NE levels in exercising subjects who were breathing either 21% $O_2$ or 100% $O_2$. They showed that hyperoxia decreased the catecholamine response to dynamic exercise. In their study, the hyperoxic subjects were
### TABLE 3

<table>
<thead>
<tr>
<th>Pressure</th>
<th>Water Temp. °C</th>
<th>Calf (Upper)</th>
<th>Calf (Lower)</th>
<th>Thigh*</th>
<th>Hand</th>
<th>Forearm</th>
<th>Arm</th>
<th>Abdomen</th>
<th>Back</th>
<th>Chest</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 ATA</td>
<td>35</td>
<td>42</td>
<td>10</td>
<td>47</td>
<td>7</td>
<td>45</td>
<td>13</td>
<td>11</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td>4 ATA</td>
<td>35</td>
<td>42</td>
<td>10</td>
<td>47</td>
<td>7</td>
<td>45</td>
<td>13</td>
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<td>24</td>
<td>28</td>
</tr>
<tr>
<td>1 ATA</td>
<td>35</td>
<td>42</td>
<td>10</td>
<td>47</td>
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<tr>
<td>1 ATA</td>
<td>35</td>
<td>42</td>
<td>10</td>
<td>47</td>
<td>7</td>
<td>45</td>
<td>13</td>
<td>11</td>
<td>24</td>
<td>28</td>
</tr>
</tbody>
</table>

| Mean Heat Flux | 10 | 12 | 38 | 13 | 17 | 11 | 8 | 7 | 4 | 3 |

 victorious threshold values for high represent the arithmetic mean of output from the two sensors.

'Two sensors were used on thigh, values for high represent the arithmetic mean of output from the two sensors.
breathing 100% O₂ at 1 ATA (i.e., 1 ATA O₂). Our subjects breathing compressed air at 4 ATA were breathing an equivalent of 0.84 ATA O₂. It is possible that the observed increase in plasma NE with hyperbaric exposure would be greater if our studies were carried out under normoxic conditions.

Stress of a general nature will cause an increase in the circulating plasma NE levels, and it may be argued that our observations are due to anxiety associated with the process of compression in the chamber. This is unlikely, because all the subjects used in this study were experienced Navy divers who had routinely undergone similar procedures in the past. Furthermore, the differences noted at 1 ATA vs. 4 ATA were maintained throughout the experiment, instead of declining as would be expected if the differences were due only to anxiety associated with compression at the start of the experiment.

Hypercapnia may be a more probable cause for the increased levels of plasma NE at 4 ATA vs. 1 ATA. Moderate hypercapnia will cause plasma levels of both NE and E to rise, the increase in NE being greater than the increase in E (27–29). Severe hypercapnia is associated with a continuing increase in plasma levels of both catecholamines, but with the E increase being much greater than the NE increase (29). Examination of the data in Fig. 4 shows that there is a significant decrease in alveolar ventilation at 4 ATA vs. 1 ATA. This decrease in alveolar ventilation at 4 ATA would cause the CO₂ tensions in the blood at 4 ATA to be greater than those at 1 ATA leading to a moderate hypercapnia. Figure 5 shows that this is indeed the case.

Examination of the data presented in Fig. 2 for E shows only minimal differences for various experimental conditions. It may be that under the conditions of our experiments, the stress due to hyperbaric exposure was not sufficient to induce a central adrenergic response but was sufficient to cause a peripheral noradrenergic response. Previous work in our laboratory showed that moderate, cool exposure caused an elevation in circulating plasma NE levels without a corresponding increase in plasma E levels (23). In dry, cool exposures Wilkerson demonstrated an increase in plasma E, NE, and cortisol at 10°C; at temperatures of 15°C and higher, plasma NE levels rose but plasma E and cortisol levels did not (14).

We did not examine the plasma for any other metabolic indicators of stress aside from the catecholamines. Though there are reports of changes in plasma cortisol levels under severe stress situations (30–33), prior work in our laboratory has revealed no significant changes in cortisol and growth hormone levels under the conditions of this study (23).

CONCLUSION

Our findings indicate that immersion in water at 25°C causes an increase in the plasma NE levels; this occurs at both 1 and 4 ATA. Independent of the effect of cool stress, hyperbaric exposure causes an increase in the level of circulating NE. The effect of pressure on the plasma NE levels is a small but definite one. This effect is statistically significant in the warm immersions. Our cool studies showed a difference in the plasma NE levels at 1 ATA vs. 4 ATA, but this difference was not statistically significant. This lack of significance in the cool studies is probably due to the greater variability in the measured NE levels in the cool studies. Alveolar ventilation at 1 ATA is greater than at 4 ATA, leading to a moderate hypercapnia at 4 ATA; this may be the primary reason for the slight increase in NE levels at 4 ATA vs. 1 ATA. Plasma NE levels are relatively nonspecific and are subject to wide variations within and between individuals. Plasma catecholamine levels are not reliable as specific indicators of stress unless a large population of subjects is used.
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catécholamines | température
---|---
stress | hypercapnée
pression | ventilation alvéolaire

REFERENCES