Increased Release of the N-Terminus of the Atrial Natriuretic Factor Prohormone with Increasing Absolute Atmospheres of Pressure in a Hyperbaric Chamber and Reversal with Oxygen Therapy*

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Increasing atmospheres of absolute pressure (ATA) on the cardiopulmonary system results in a marked diuresis. The present investigation was designed to determine if the diuresis observed with increasing ATA is associated with increased release of the N-terminus of the atrial natriuretic factor (ANF) prohormone that contains two potent diuresis-producing hormones consisting of amino acids (aa) 1-30 (pro ANF 1-30; long-acting sodium stimulator) and aa 31-67 (pro ANF 31-67; vessel dilator) of this 126 aa prohormone. Seven healthy volunteers (mean age, 31 years) had the circulating concentration of the N-terminus of the ANF prohormone evaluated at 1, 2, and 3 ATA in a monoplace hyperbaric chamber by two specific and sensitive radiolmmunoassays that immunologically recognize (1) the whole 98 aa N-terminus and (2) the midportion of the N-terminus consistent with aa 31-67 (pro ANF 31-67). With increasing ATA from 1 (sea level) to 2 (equivalent to 33 feet of sea water), the circulating concentrations of both the whole N-terminus and pro ANF 31-67 increased threefold. At 3 ATA (66 feet of sea water), their circulating concentrations increased sixfold over their concentrations, at 1 ATA. With the addition of 100 percent O2 while at 3 and 2 ATA, the circulating concentrations of both the whole N-terminus and pro ANF 31-67 immediately decreased to their prehyperbaric ATA levels and remained there with further decompression to 1 ATA and removal of O2 supplementation. The increased circulating concentration of the N-terminus of the ANF prohormone containing two peptides with potent diuretic effects during increasing atmospheres of absolute pressure may help to explain the diuresis that has been observed with increasing ATA.

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\[ \text{ATA} = \text{atmospheres of absolute pressure; ANF = atrial natriuretic factor; aa = amino acid} \]

Increasing atmospheres of absolute pressure (ATA) on the cardiopulmonary system as observed in hyperbaric chambers or with deep sea diving is associated with a marked diuresis.1-3 This diuresis that is sustained in prolonged dives has become a major problem for divers.1-3 Increasing ATA has recently been demonstrated to produce a linear increase in atrial natriuretic factor (ANF), a new cardiac hormone with potent natriuretic and diuretic properties.6 This linear increase in ANF, the 28 amino acid (aa) C-terminus of the 126 aa prohormone, may partially explain the diuresis that has been observed with increasing atmospheres of pressure.6 The N-terminus (aa 1-98) of this prohormone also contains peptides with potent diuretic, natriuretic, and vasodilatory properties.7,9 These N-terminal peptides consisting of aa 1-30 (pro ANF 1-30; long-acting sodium stimulator) and aa 31-67 (pro ANF 31-67; vessel dilator) may also be involved in causing this diuresis since the N-terminus circulates normally in man.10-16 The N-terminus, in general, increases in the circulation in response to the few stimuli that increase the C-terminus.12,18,21 Thus, stimuli such as central hypervolemia12,15 and rapid cardiac pacing21 release the N-terminus and C-terminus of the ANF prohormone simultaneously. Whether increasing ATA does increase the circulating concentration of the N-terminus of the ANF prohormone with its diuresis producing peptides is unknown. The present investigation was designed to determine the response to increasing ATA in a monoplace hyperbaric chamber on the circulating concentration of the N-terminus of the ANF prohormone using two specific radioimmunoassays, one of which immunologically recognizes the whole 98 aa N-terminus (pro ANF 1-98). The second radioimmunoassay evaluates the circulating concentration of the

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midportion of the N-terminus (pro ANF 1-30) that
circulates as a distinct entity after being proteolytically
claveed from the rest of the N-terminus.

Oxygen is often added to hyperbaric chambers to
help in the healing of burn patients or to treat
decompression sickness.\textsuperscript{22} To determine the effect of
hyperbaric O\textsubscript{2} that patients may be given for wound
healing, burns, or for treatment of decompression
sickness, the circulating concentrations of the whole
N-terminus and midportion of the N-terminus were
monitored after the addition of 100 percent O\textsubscript{2} to the
hyperbaric chambers while the volunteers were at 1
atmosphere of pressure (ie, sea level), 2 ATA (simulated
depth of 33 feet of sea water), and 3 ATA (66 feet of
sea water).

**Experimental Methods**

**Subjects**

Seven volunteers (three men and four women) with diving
experience of four to 14 years for the men and from one to seven
years for the women had the plasma concentration of the
N-terminus of the ANF prohormone (pro ANF 1-98) and pro ANF 31-
67 measured at 1, 2, and 3 ATA. The average age of these seven
volunteers was 31 years, with the men being 23, 29, and 41 years
old and the women being 27, 31, 32, and 33 years old. All seven
volunteers were normotensive with heart rates of 66 to 80 beats per
minute. Their respirations varied from 10 to 16 per minute. None of
the volunteers had any known medical disease and none of these
volunteers was receiving any medication. There was no discernible
evidence of renal dysfunction with the serum creatinine being
below 1.5 mg/dl in each of these seven subjects. Informed consent
was obtained from these seven volunteers after the nature and
possible consequences of the study had been fully explained. This
investigation was approved by the Human Use Committees of both
the University of Arkansas for Medical Sciences and the John L.
McClellan Memorial Veterans Hospital.

**Hyperbaric Protocol**

Each of the divers was placed in a monoplace hyperbaric dry
chamber (Sechrist Model 2500B, Anaheim, CA). A No. 18 heploclip
(Jelco) was placed in the brachial vein of each subject. This heplock,
in turn, was attached to an intravenous tubing through a micro
outlet in the hyperbaric chamber for venous sampling from outside
the chamber during the various atmospheric changes. Once the
volunteer had entered the chamber and the chamber was sealed,
the first plasma sample was obtained at room air. Compression was
then begun at a rate of 5 lb/min (ppm). After stabilizing at 2
atmospheres for 5 minutes, another blood sample was obtained.
Likewise, after stabilizing at 5 atmospheres for 5 minutes, a third
blood sample was obtained. Each of the volunteers was then
taken from their room air environment within the hyperbaric
chamber to 100 percent O\textsubscript{2}. Following ten minutes of 100 percent
O\textsubscript{2} at 3 ATA, another plasma sample was drawn. Each volunteer
then began decompression sequence of 10 minutes at 2 ATA with
100 percent O\textsubscript{2} followed by another blood sample and then
decompression to surface air. After 30 minutes at surface atmos-
pheric pressure, a final blood sample was obtained for comparison
of the circulating concentrations of pro ANFs 1-98 and 31-67 with
and without 100 percent O\textsubscript{2}.

**Radioimmunoassays for Pro ANF 1-98, Pro ANF 31-67, and ANF**

Radioimmunoassays to measure the N-terminus of the ANF
prohormone were devised to aa 1-30 and 31-67 of the 126-aa
prohormone, while the C-terminal assay measures aa 99-126 of the
prohormone, ie, ANF, as described previously by our laboratory.\textsuperscript{11,17}
Our pro ANF 1-30 radioimmunoassay recognizes a component in
plasma of approximately 10,000 MW as characterized by C-50
Sephadex gel permeation chromatography that is consistent with
the whole N-terminus (ie, aa 1-98), but without the C-terminus
attached to it.\textsuperscript{11} The pro ANF 31-67 radioimmunoassay recognizes
mainly in plasma a component of the N-terminus of approximately
3,900 MW, which is consistent with it measuring pro ANF 31-67
(3,878 MW).\textsuperscript{11}

For the radioimmunoassay of ANF, pro ANF 1-98, and pro ANF
31-67, the extracted plasma was first reconstituted in 100 \muL of 0.1
mol/L phosphate buffer (pH 7.4), containing 0.5 mol/L NaCl, 0.1
percent bovine serum albumin, 0.1 percent Triton X-100, and 0.01
percent NaN\textsubscript{3}. To the redissolved sample, 100 \muL (0.03 mg) of rabbit
IgG plus 20 \muL of the respective antisera were added and incubated
for 24 hours. Then 100 \muL of the \textsuperscript{125}I-labeled peptides (10,000 cpm)
were added and incubated for 18 hours at 4°C. The precipitation of
the antibody-bound tracer was accomplished by adding 100 \muL of
goat anti-rabbit globulin after the above-described 18-hour period
and incubating this mixture for 2 hr at room temperature. Each tube
was then centrifuged at 3,000 g for 20 minutes. The supernatant
was aspirated and the pellet counted in a gamma counter. All
determinations were performed in triplicate. The intraassay coeffi-
cient of the variation for pro ANF 1-30, 31-67, and ANF radioim-
monoassays were 4.8, 5.3, and 5.7 percent, respectively. The
interassay coefficient of variation was 8 percent for both pro ANF
1-30 and 31-67 radioimmunoassays whereas ANF radioimmunoas-
says' interassay variation was 6.9 percent.

Recovery was examined by adding synthetic unlabeled pro ANF
1-30, pro ANF 31-67, and ANF at 100, 200, and 400 pg/ml to pooled
human plasma. Recovery for pro ANF 1-30 was 83.5±\textsubscript{4} 13.2 percent
(SD) and pro ANF 31-67 recovery was 100.9±\textsubscript{4} 8.9 percent (SD).
Recovery of ANF was 92±\textsubscript{4} 11 percent (SD).

The respective IC\textsubscript{50} were 150, 120, and 11 fmol per tube while
the lowest detectable concentrations were 40, 35, and 1.4 fmol for
pro ANFs 1-30, 31-67, and ANF, respectively. Serial dilution of
pooled plasma has revealed excellent parallelism of standard and
unknown in these assays. Reverse phase high-pressure liquid
chromatography using Nova pak C-18 (5 micron) cartridge columns
revealed that the pro ANF's 1-30, 31-67, and 99-126 (ANF) measured
were authentic.

In the presentation of the data, mean values are followed by the
standard error of the mean as an index of dispersion. Data were
evaluated statistically by analysis of variance (ANOVA). Differences
with p<0.05 were considered significant.

**Results**

The circulating concentration of the whole N-ter-
minus (immunoreactive pro ANF 1-98) of the ANF
prohormone increased linearly with increasing ATA
from 1 to 3 ATA in each of the seven individuals. (Fig
1). There was a threefold increase in the N-terminus
of the ANF prohormone with 2 ATA in the hyperbaric
chamber (Fig 1). This significant (p<0.01; ANOVA)
increase at 2 ATA is equivalent to pressure exerted in
a dive of 33 feet of sea water. With increasing the ATA
to 3 (equivalent to a depth of 66 feet of sea water),
the circulating concentration of the N-terminus of the
ANF prohormone increased further to become sixfold
higher than its concentration at 1 ATA (p<0.01;
ANOVA). The circulating concentration of pro ANF 1-
98 at 3 ATA was also significantly higher (p<0.05;
ANOVA) than its concentration at 2 ATA. This increase
in pro ANF 1-98 was linear with increasing atmos-

Hyperbaric Chamber and Oxygen Therapy (Rico et al)
Figures of increasing atmospheres of absolute pressure (ATA) on the circulating concentration of the N-terminus (amino acids 1-98; pro ANF 1-98) of the 126 amino acid atrial natriuretic factor (ANF) prohormone. The increases in the circulating concentrations of pro ANF 1-98 with increasing ATA from 1 ATA (sea level) to 2 ATA (equivalent to 33 feet of sea water) and from 2 ATA to 3 ATA (66 feet of sea water) in a monoplace hyperbaric chamber in seven volunteers breathing air was significant at p<0.05 when evaluated by the analysis of variance (ANOVA). The addition of 100 percent O₂ to the hyperbaric environment at 3 ATA produced a significant (p<0.05; ANOVA) decrease in the circulating concentration of pro ANF 1-98 in all seven individuals. With further decompression to 2 and 1 ATA while receiving 100 percent O₂, the circulating concentration of pro ANF 1-98 remained significantly (p<0.05; ANOVA) decreased compared with its pre-O₂ concentration. There was no rebound increase in the whole N-terminus of the ANF prohormone during a 30-minute period after discontinuing O₂ supplementation.

The midportion of the N-terminus (ie, pro ANF 31-67) that circulates as a distinct entity after being proteolytically cleaved from the N-terminus of the ANF prohormone was, likewise, significantly higher (p<0.05; ANOVA) in the circulation at 2 ATA compared with 1 ATA (Fig 2). With increasing the absolute atmospheric pressure to 3 ATA, there was a further significant increase (p<0.05; ANOVA) in the circulating concentration of the midportion of the N-terminus in these seven healthy individuals compared with that observed at 2 ATA (Fig 2). The increase in pro ANF 31-67 with increasing ATA thus followed a pattern similar to that of the whole N-terminus in these seven volunteers as one can observe by comparing Figures 1 and 2.

The addition of 100 percent O₂ to the hyperbaric chamber while these seven volunteers were at 3 ATA (ie, simulated depth of 66 feet) resulted in a dramatic decrease in the circulating concentration of the N-terminus of the ANF prohormone (Fig 1). The circulating N-terminus concentration decreased from its sixfold increased concentration at 3 ATA to that of a person at sea level (Fig 1). Pro ANF 1-98 remained in the range of persons at sea level during decompression with O₂ supplementation from 66 to 33 feet and with further decompression to sea level (Fig 1). There was no rebound increase in the N-terminus of the ANF prohormone with discontinuing O₂ supplementation at sea level during a 30-minute post-O₂ observation period.

Oxygen supplementation (100 percent) to the hyperbaric chamber also caused an immediate decrease in the circulating concentration of pro ANF 31-67 while these seven individuals were at 3 ATA (Fig 2). With decompression from 3 ATA to 2 ATA and further reduction to 1 ATA with supplemental O₂, pro ANF 31-67 remained at its circulating concentration observed in healthy persons at 1 ATA breathing air outside of a hyperbaric chamber (Fig 2). With discontinuation of O₂ supplementation at 1 ATA, there was no rebound in the circulating concentration of pro ANF 31-67 during a 30-minute post O₂ observation period.

**Discussion**

In the present investigation, there was a linear
increase in the circulating concentrations of both the whole N-terminus and the midportion of the N-terminus (pro ANF 31-67) of the ANF prohormone with increasing absolute atmospheres of pressure. Since pro ANF 31-67 and pro ANF 1-30 from the N-terminus of this prohormone have potent diuretic properties, the increased concentrations of the whole N-terminus and pro ANF 31-67 with increasing ATA suggests that these peptide hormones in addition to ANF (aa 99-126; C-terminus of the prohormone) described previously may contribute to the diuresis that has been observed with increasing atmospheres of pressure. With similar ATA (4 ATA), a sustained diuresis is well documented. Other hormones that affect water and salt metabolism have previously been investigated and found not to be the cause of the diuresis observed with increasing atmospheres of pressure. Thus, there was little change in antidiuretic hormone (vasopressin) or plasma aldosterone with a saturation dive. The only hormone shown to increase in previous studies of increasing ATA has been plasma renin activity. One would not expect that a plasma renin activity increase would cause any diuresis since increasing plasma renin activity with a subsequent increase in angiotensin II decreases renal blood flow and decreases glomerular filtration rate resulting in sodium retention and decreased urine flow rather than diuresis and natriuresis.

With respect to the cause of the release of the N-terminus of the ANF prohormone with increasing ATA, there are at least two possibilities. One, the acute compression of the chest with 14.7 lb of pressure per square inch being added for every 1 ATA contributes to a blood volume redistribution with a resultant central hypervolemia due to hydrostatic forces. Central hypervolemia has recently been demonstrated to be a strong stimulus to the release of both the C-terminus and N-terminus of the ANF prohormone. The hydrostatic forces associated with increasing ATA appear to be major contributors to the release of the N-terminus and C-terminus of the ANF prohormone, for although a second possible contributor, hypoxia, has been shown to release ANF by itself, alveolar PO₂ increases even with decreasing lung volume associated with increasing atmospheres of pressure. This central hypervolemia is probably also partially
due to peripheral vasoconstriction noted in tissue hyper-O_2 states.\textsuperscript{27} Air breathing at 2 ATA equals 40 percent tissue O_2 at surface while 3 ATA is equivalent to 60 percent tissue O_2 at surface.

Hyperbaric O_2 therapy rapidly decreased the N-terminus of the ANF prohormone in the present investigation when added at 2 and 3 ATA to volunteers who had previously been breathing air. The addition of O_2 at 1 ATA caused no change in the circulating concentration N-terminus of the ANF prohormone. Whether the rapid reduction in the N-terminus of the ANF prohormone seen in the present investigation or the reduction in the C-terminus seen previously with hyperbaric O_2 therapy\textsuperscript{6} contributes to prevention of decompression sickness could not be determined from the present investigation. It should be mentioned that 100 percent O_2 at 2 to 3 ATA for longer periods of time could cause O_2 toxicity, which theoretically might also decrease the circulating N-terminal and C-terminal ANF prohormone concentrations, but with the short period of 100 percent O_2 used in the present investigation, O_2 toxicity would be very unlikely.\textsuperscript{28} There were no symptoms of O_2 toxicity in any of the seven volunteers in the present investigation.

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