Volemic Status Influences the Response of Plasma Atrial Natriuretic Factor to Positive Airway Pressure*

Philippe Beuret; François Feihl, MD; Jürg Nussberger, MD; Hans-Rudolph Brunner, MD; and Claude Perret, MD, FCCP

**Study objective:** To evaluate interactive effects of volemic status and positive end-expiratory pressure (PEEP) on the plasma levels of atrial natriuretic factor (ANF) in assist-controlled mechanical ventilation (MV).

**Design:** Three successive protocols applied in randomized order to each participant.

**Setting:** Clinical investigation laboratory.

**Participants:** Twenty-one young, healthy adults.

**Interventions:** The three protocols were as follows: (1) MV+PEEP, normovolemia; (2) MV+PEEP, hypervolemia; and (3) spontaneous breathing (SB), hypervolemia. In protocols 1 and 2, a preliminary period of SB lasting 2 h was followed by MV alone (0.5 h), MV+20 cm H2O PEEP (1 h), and a recovery period of SB (1.5 h). Hypervolemia was induced by the continuous IV infusion of 3 L of 0.9% NaCl in 5 h (protocols 2 and 3).

**Measurements and results:** Heart rate, BP, and the plasma levels of immuno-reactive ANF and catecholamines were measured serially. During hypervolemia, ANF significantly decreased when PEEP was added to MV (protocol 2: from 31.1±2.7 to 20.7±1.5 fmol/mL; p<0.01). This did not occur in normovolemia (protocol 1: from 20.0±2.0 to 16.7±1.2 fmol/mL; p=NS). The different effects of MV+PEEP in normovolemia and hypervolemia were not related to differences in circulating catecholamine levels.

**Conclusions:** These results demonstrate for the first time (to our knowledge) that volemic status modulates the response of plasma ANF to PEEP in humans. The role of ANF in the water and salt retention induced by MV with PEEP might be limited to hypervolemic conditions.

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**Key words:** atrial natriuretic factor; positive pressure respiration; respiration, artificial

**Abbreviations:** ANF=atrial natriuretic factor; EPI=epinephrine; f=respiratory rate; MV=positive-pressure mechanical ventilation; NE=norepinephrine; PEEP=positive end-expiratory pressure; SB=spontaneous breathing; Vr=tidal volume

Atrial natriuretic factor (ANF) is released from secretory granules located in atrial myocytes.1 It has been shown to induce diuresis, natriuresis, vasorelaxation, and to inhibit renin and aldosterone secretion.2,3 Atrial distention appears to be the predominant stimulus triggering the release of ANF.3 It has also been reported that phenylephrine, epinephrine (EPI), angiotensin II, and vasopressin may increase ANF secretion in vitro.3

Positive-pressure mechanical ventilation (MV) with positive end-expiratory pressure (PEEP) is a widely used procedure in the treatment of acute respiratory failure. MV with PEEP reduces cardiac output through various mechanisms,5,6 moreover, it often induces antidiuresis and fluid retention.7,8 These side effects are amplified when circulating volume is low or normal.9 Their exact mechanisms are still controversial.8,10-13 It has been proposed that the antidiuresis induced by PEEP is mediated in part by a decreased ANF secretion secondary to a reduction in atrial transmural pressure.10,14-15 Indeed, there are several animal16,17,19 and human10,14,15,20 studies reporting concomitant decreases of urine output, urinary sodium excretion, and plasma levels of ANF in response to the institution of either MV+PEEP10,14,16,17,19,20 or continuous positive airway pressure.15 However, several studies failed to document parallel changes of renal function and plasma ANF levels on variations of mean intrathoracic pressure.11,13,21-23 Most of this latter work13,21-23 pertains to data obtained in normovolemic conditions, while all but one16 of the studies reporting a depressor
effect of MV+PEEP on plasma ANF were conducted in the presence of volume expansion. This raises the possibility that volemic status modulates the response of ANF secretion to positive intrathoracic pressure, a hypothesis supported by recent animal data \(^{13}\) but never tested in humans (to our knowledge).

The aim of the present study was to gain further insights into the interactions of PEEP, ANF secretion, and volemic status in human subjects. Specifically, we sought to determine the effects of high levels of PEEP (20 cm H\(_2\)O) on the plasma levels of ANF in normal subjects and to compare these effects in two different situations: normovolemia and hypervolemia.

**Materials and Methods**

Twenty-one healthy volunteers (10 men and 11 women) aged 19 to 41 years (mean, 26.4 years) were studied. None of them was taking any medications. All gave their informed consent and the protocol was approved by the local ethics committee. The subjects were selected after a preliminary evaluation of their ability to tolerate MV in assist/control mode with 20 cm H\(_2\)O PEEP while nearly relaxing their respiratory muscles.

**MV and Monitoring**

MV was performed via a tightly fitting face mask using a volume-controlled ventilator (Servo 900 Siemens; Elema, Sweden). The ventilator settings were predetermined (assist/control mode; tidal volume [VT], 12 mL/kg; respiratory rate [f], 12/min; I/E ratio, 25%; inspiratory pause time, 10% of the total respiratory cycle; fraction of inspired oxygen, 0.21). VT and f were then adapted for maximal subject comfort and an end-tidal PCO\(_2\) (78345A UL monitor; Hewlett-Packard; Palo Alto, Calif) between 30 and 40 mm Hg. Thereafter, end-tidal PCO\(_2\) was maintained in this range by adjusting f if necessary, while VT remained strictly constant. The airway pressure was measured at the mouth, monitored on a video display and recorded on paper (Hewlett-Packard 78172A). The pressure transducer (Deseret Medical Inc; Sandy, Utah) was calibrated using a water column. The PEEP level was assessed from the strip chart recording of airway pressure. Heart rate was determined from a three-lead ECG signal. At 30-min intervals, systolic and diastolic BP (Korotkoff V) was measured by sphygmomanometry.

Comfort during the experiment was enhanced by having subjects listen to music of their choice through headphones when desired. As assessed from the airway pressure waveform, total or nearly total relaxation of respiratory muscles was achieved during the whole course of MV in all instances.

**Protocols**

Each subject underwent three different protocols, each beginning at 8 am and lasting 5 h. Protocols were separated by at least 72 h and at most 1 week. All protocols were performed with the subject in a strictly supine position. Two plastic cannulas were placed into the antecubital veins, one for blood sampling, and one for IV fluid administration (see below). The three protocols, schematically displayed in Figure 1, were applied in random order. In protocol 1 (MV+PEEP, normovolemia), 2 initial hours (from T=-120 to T=0) of spontaneous breathing (SB) were followed by 30 min of MV with zero PEEP (from just after T=0 up to T=30), then by 60 min of MV with 20 cm H\(_2\)O PEEP (from just after T=30 up to T=90), and finally by 90 min of recovery under spontaneous ventilation (recovery SB, from just after T=90 up to T=180). In protocol 2

![Figure 1](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21731/ on 06/26/2017)
(MV+PEEP, hypervolemia), ventilation followed the same pattern as in protocol 1, and volume expansion was added in the form of a continuous IV infusion of 0.9% NaCl at a rate of 750 mL/h during the first 2 h (rapid volume expansion) and 500 mL/h during the last 3 h (slow volume expansion). In protocol 3 (SB, hypervolemia), ventilation was spontaneous throughout and volume expansion was performed as described in protocol 2. The volunteers were told to refrain from salt-rich food and not to drink during, respectively, 48 and 12 h prior to the experiments. Three hundred milliliters of fluid was given to the subjects 1 h before the start of the study.

In these healthy, relaxed subjects, the compliances of the lung and chest wall were presumably equal. In such conditions, the applied PEEP was expected to raise intrathoracic pressure by about 10 cm H₂O (ie, one half of 20) at the end of expiration.24

ANF Measurements

Great care was taken to keep a strict supine position throughout the experiments (ie, from T=-120 to T=180, Fig 1) since the plasma level of ANF is dependent on body position.25 Blood samples for ANF assay were drawn at T=-60, 0, 30, 60, 90, 120, and 180 min (Fig 1). The samples (10 mL) were collected into prechilled glass tubes containing EDTA (ethylene diamine tripotassium tetraacetate) and immediately centrifuged at 4°C for 10 min. Plasma aliquots were flash frozen in liquid nitrogen and stored at −70°C until assay. The plasma levels of immunoreactive ANF were measured by a highly sensitive radiomunnoassay after extraction on bonded-phase silica. The detailed procedure has been described elsewhere.26 As shown in this previous report, its accuracy and precision were sufficient to detect changes in plasma ANF levels well within the normal physiologic range (6 to 34 fmol/mL): the detection limit was 0.14 fmol per assay tube, corresponding to 1.1 fmol/mL plasma ANF; the interassay coefficient of variation was less than 13% for ANF concentrations less than 25 fmol/mL and less than 7% for higher concentrations. Accuracy of the assay was shown by reaching median values for low, intermediate, and high plasma ANF concentrations of an international standardization study organized by the World Health Organization.20,27 Under the storage conditions used, the measured plasma concentrations of ANF remained stable for more than 1 year.

Catecholamine Measurements

Following the same sampling procedure as for ANF measurement but using glass tubes containing heparin, 5 mL of blood was also collected in the last 12 of the 21 volunteers at T=-60, T=90, and T=180 (Fig 1) to determine plasma EPI and norepinephrine (NE) by a radioenzymatic assay.25

Data Analysis

All variables were analyzed identically. The time course during each protocol and the differences between protocols at each experimental time were evaluated by means of analysis of variance for repeated measures. When the overall F value was significant (p<0.05), further tests were carried out using Dunnett (within-protocol comparisons of different times to a control time that was T=-60 for catecholamine measurements and T=0 otherwise) or Newman-Keuls procedure (between-protocol comparisons at specific times). In the cases of ANF and EPI, a variance-stabilizing logarithmic transform was applied prior to analysis. Data are summarized as the mean±SEM.

RESULTS

ANF

Figure 2A (top) shows the time course of ANF from T=-60 to T=180 during each of the three study protocols. In protocol 1, ANF was stable in the last hour preceding the initiation of MV, ie, between T=-60 (19.6±2.0 fmol/mL) and T=0 (20.0±1.8 fmol/mL). This stability of measurements taken in identical conditions illustrates the reliability of the ANF assay. Af-
after 30 min of MV alone (T=30) and 1 h of MV+PEEP (T=90), the plasma levels of ANF were, respectively, 18.8±1.2 and 16.7±1.2 fmol/mL, neither significantly different from the value at T=0. Thus, neither MV alone nor MV+PEEP had any detectable effect on ANF plasma level in conditions of normovolemia.

In protocol 2, ANF increased from 22.4±2.0 fmol/mL at T=−60 to 31.1±2.7 at T=0 (p<0.01) reflecting volume expansion. Despite further volume load, ANF stabilized with the addition of MV (27.1±2.5 fmol/mL at T=30) and then significantly decreased with the addition of PEEP (20.7±1.5 fmol/mL at T=90, −33.4% as compared to T=0; p<0.01).

In protocol 3, ANF increased significantly from T=−60 to T=0, that is during the rapid volume expansion (19.4±2.0 to 35.7±3.2 fmol/mL; p<0.01). A further increase later on during the slow volume expansion (to 41.5±5.8 fmol/mL) did not reach statistical significance.

**Catecholamines**

Plasma EPI remained within the normal range (5 to 150 pg/mL) throughout the entire study. It did not change during hypervolemia in the absence of MV. With MV+PEEP, however, EPI did increase both under normovolemic and hypervolemic conditions (Fig 2B, center); during protocol 1, EPI increased from T=−60 to T=90 (20.6±3.1 to 78.3±11.4 pg/mL; p<0.01) and then returned close to the initial value at T=180. During protocol 2, EPI increased from T=−60 to T=90 (18.1±3.4 to 58.8±10.1 pg/mL; p<0.01) and then returned to baseline. There was never a significant difference in EPI levels between the protocols 1 and 2, but there was a trend for EPI to increase more with PEEP in normovolemic (fourfold) than in hypervolemic conditions (threelfold).

There were no statistically significant changes in NE, which remained within the normal range (50 to 400 pg/mL) during the three protocols; however, mean plasma NE tended to decrease during volume expansion in the absence of PEEP (protocol 3), but PEEP blunted this trend (protocol 2) and actually reversed it in the absence of volume load (protocol 1) (Fig 2C, bottom).

**Heart Rate and Arterial BP**

As shown in Figure 3, MV+PEEP significantly increased heart rate as well as systolic and diastolic arterial BP in protocol 1 (p<0.01); in contrast, during hypervolemia in protocol 2, the heart rate response to MV+PEEP was blunted and statistically not significant, while BP increased to slightly higher values than during normovolemia. In protocol 3 (without MV), neither arterial BP nor heart rate changed significantly.

**Discussion**

The present study confirms that in hypervolemic normal conscious subjects, MV with PEEP decreases the plasma levels of ANF, while increasing the plasma...
EPI levels and arterial BP. In the same subjects, PEEP administered without concomitant volume expansion does not change ANF. By contrast, volemic status per se has minimal influence on the time course of plasma catecholamines and arterial BP during these experiments. To our knowledge, the present study is the first to demonstrate that volemic status modulates the response of plasma ANF to PEEP in humans.

As reviewed in the introduction, conflicting data have been reported concerning the influence of positive intrathoracic pressure on ANF secretion; while caution should be exercised in comparing studies that used different ANF assays or various PEEP levels and were conducted in animals, healthy volunteers, or patients in the ICU, one possible cause for discrepancies could relate to differences in volemic conditions. This is supported by recent data showing in five long-term instrumented conscious dogs that MV with 20 cm H2O PEEP might depress the plasma levels of ANF in presence, but certainly not in absence of massive volume expansion (IV crystalloids, 120 mL/kg of body weight over 4 h).13 Our data extend these observations to human subjects exposed to this amount of PEEP while receiving either no or moderate volume expansion (approximately 40 mL/kg of body weight).

Because atrial distention, right or left, is the main stimulus for ANF secretion,2-4 it would have been of critical importance to know the influence of PEEP on atrial volumes. The accurate measurement of these volumes is technically difficult and not feasible in healthy volunteers. In response to PEEP of 15 to 16 cm H2O, right and left atrial volumes assessed by transesophageal echocardiography decreased in mechanically ventilated patients with either normal28 or abnormal29 respiratory mechanics. To the best of our knowledge, the response of atrial volumes to the higher PEEP (ie, 20 cm H2O) used in the present study has not been described; data concerning effects of such high PEEP on right and left ventricular volumes3,6 cannot be extrapolated to the atria, if only because of the known spatial inhomogeneities of compressive forces exerted on the heart by the overinflated lungs.6

In the present study, MV+PEEP failed to modify the plasma level of ANF in normovolemia. This would be expected if the atria were unstressed, so that changes in atrial volume did not modify atrial wall stress. That the right atrium can actually be unstressed has been suggested by the observation of near zero right atrial transmural pressure in humans.30 However, this remains controversial31 and is not necessary to explain our findings. Indeed, during progressive volume expansion in the dog, right atrial transmural pressure must rise above a threshold of 3 to 4 mm Hg before any increase in plasma ANF is observed.32 The different responses of plasma ANF according to volemic status (Fig 1) could thus depend on atrial stretch being above some threshold in hypervolemia, but not in normovolemia.

In accordance with the concept that the response of ANF secretion to PEEP is in some way modulated by the baseline degree of atrial distention, the depression of plasma ANF on institution of PEEP is less marked in anesthetized dogs before than after the experimental induction of acute left ventricular failure,33 which is expected to produce atrial dilatation. Along the same line, nasal continuous positive airway pressure (10 cm H2O) has been recently shown to decrease the circulating level of ANF in patients with chronic heart failure.34

MV+PEEP induced sympathoadrenergic activation, as attested by increased plasma EPI levels (Fig 2), BP, and heart rate (Fig 3). This response to positive airway pressure has been consistently observed in healthy volunteers14,23,33 but not in patients,10,11,14,18,20,36 a difference possibly related to a greater discomfort induced by MV+PEEP when administered by a face mask in the absence of sedation. It cannot be formally excluded that sympathoadrenergic activation influenced our results. The more prominent increase in heart rate (Fig 3) and the trend of both E and NE to augment more with PEEP in normovolemic conditions (Fig 2) would be consistent with the hypothesis that hypervolemia blunted the sympathoadrenergic response to PEEP. Such a blunted response has been demonstrated experimentally37 and could represent the removal of a stimulus for ANF secretion. However, this possibility appears unlikely for two reasons. First, the influence of hypervolemia on the responses of plasma catecholamine levels (Fig 2), heart rate, BP (Fig 3) to MV+PEEP was minimal, if at all present. Second, direct secretagogue effects of neurotransmitters or circulating catecholamine levels on atrial myocytes, although suggested, have not been convincingly demonstrated.2,3,38

One should be cautious about extending our results to the clinical situation. In many patients receiving MV, PEEP is both lower than used in the present study and less well transmitted to intrathoracic pressure due to abnormally stiff lungs. Moreover, in addition to volemic status, the concomitant presence and severity of right and/or left ventricular dysfunction may also influence atrial volume and stretch. In a series of patients receiving MV for acute respiratory failure, the drop in plasma ANF level induced by a PEEP of 15 cm H2O was variable, being more marked in the subjects with the highest ANF levels prior to PEEP.14 This suggests modulation of the ANF response to PEEP by the baseline degree of atrial stretch, assuming that the
trigger mechanisms for the release of ANF are not radically altered in patients.

In conclusion, among the different mechanisms involved for explaining the antidiuresis and sodium retention frequently observed during MV with PEEP, a reduced ANF secretion has been proposed. Our results show that such a mechanism, if operative, is clearly dependent on volemic status. If these data obtained in normal volunteers are also valid for patients, one can assume that, in absence of cardiac dysfunction, MV promotes sodium and water retention by decreasing ANF secretion only in hypervolemic subjects.

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