Reciprocal Regulation of Plasma Apelin and Vasopressin by Osmotic Stimuli

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ABSTRACT

Apelin is a neuropeptide that co-localizes with vasopressin (AVP) in magnocellular neurons and is involved in body fluid homeostasis. Osmotic stimuli have opposite effects on the regulation of apelin and AVP secretion in animal models, but whether this is true in humans is unknown. This study investigated the relationship among osmolality, apelin, and AVP in 10 healthy men after infusion of hypertonic saline or loading with water to increase and decrease plasma osmolality, respectively. Increasing plasma osmolality was accompanied by a parallel, linear increase in plasma AVP concentration and by a decrease in plasma apelin concentration. In contrast, decreasing plasma osmolality by water loading reduced plasma AVP concentration and rapidly increased plasma apelin concentration. These findings suggest that regulation of apelin secretion contributes to the maintenance of body fluid homeostasis.


The osmotic pressure of body fluids is maintained within a remarkably narrow range in healthy adults. Body fluid homeostasis depends on neuronal pathways bearing very sensitive osmoreceptors,1 located along the lamina terminalis, including the circumventricular organs, such as the subfornical organ and the organum vasculosum of the lamina terminalis as well as the median preoptic nucleus.2 The subfornical organ and organum vasculosum of the lamina terminalis are neuronally interconnected with each other as well as with the median preoptic nucleus and the hypothalamic paraventricular and supraoptic nuclei.3 These neuronal pathways convert small changes in osmolality into a neuronal signal to neurons that influence sensations of thirst and systemic arginine vasopressin (AVP) release,2 thereby adjusting the intake or output of water to counteract changes in solute concentration.4,5

A recently discovered peptide, apelin, may also play a major role in the maintenance of body fluid homeostasis. Apelin, initially isolated from bovine stomach extracts,6 is the endogenous ligand of the human orphan G protein–coupled receptor APJ (putative receptor protein related to the angiotensin receptor AT1).5,7 It is a 36–amino acid peptide (apelin 36) derived from a single 77–amino acid precursor, proapelin.6,8,9 Proapelin has a fully conserved C-terminal 17–amino acid sequence, apelin 17 (K17F), including the pyroglutamyl form of apelin 13 (pE13F). K17F and pE13F both are present in rat brain and plasma,10 and apelin 36 is present in testis and uterus.11 All peptides exhibit a high affinity for the human8,12,13 and the rat apelin receptors.14 Apelin possesses various cardiovascular functions (for reviews,15–17). Apelin and its receptor have been detected in the endothelial cells of large conduit arteries, coronary vessels, and the endocar-
Apelin and its receptor both are widely distributed in the brain but are particularly abundant in the supraoptic nucleus and paraventricular nucleus, where they co-localize with AVP in magnocellular neurons. Intracerebroventricular injection of K17F inhibits the typical phasic firing pattern of AVP neurons in lactating rats, resulting in decreased systemic AVP release and increased aqueous diuresis. Moreover, water deprivation in rats while increasing systemic AVP release and causing depletion of hypothalamic AVP stores decreases plasma apelin concentrations and results in hypothalamic accumulation of the peptide. Thus, the two peptides are conversely regulated to optimize AVP release into the blood circulation and prevent additional water loss through the kidney. Whether such opposite regulation of apelin and AVP secretion in response to osmotic stimuli is found in humans remains unknown.

The aim of this clinical investigation was to examine the relationship between plasma osmolality, plasma apelin, and plasma AVP in healthy adults in various states of hydration. Hydration was modified by administration of a hypertonic saline infusion and by water loading to increase and decrease plasma osmolality, respectively.

RESULTS

Characterization of the Molecular Forms of Apelin Present in Human Plasma

Apelin immunoreactivity (apelin-IR) was resolved as two major components eluting in fraction 28 and fractions 31 to 32 and a minor component eluting in fraction 40 (Figure 1). Calibration of the C4 column with apelin fragments revealed that the prominent immunoreactive compounds co-eluted with pE13F and K17F, respectively, and that the apelin-IR present in fraction 40 exhibited the same retention time as synthetic apelin 36 (Figure 1).

Plasma Apelin Levels in Physiologic Unrestricted Water Intake Conditions on Day −1

At 8:00 a.m. on day −1, the plasma apelin concentration in 10 healthy individuals was 477 ± 167 fmol/ml; plasma osmolality (286 ± 4 mOsm/kg H2O) and plasma AVP concentration (median [interquartile range (IQR)] 1.8 [1.4 to 3.0] fmol/ml) both were within the physiologic range. Plasma osmolality, apelin, AVP, and apelin/AVP ratio remained stable between 8:00 a.m. and 8:00 p.m. with no significant changes associated with the 12-h overnight fluid and food restriction (Table 1). The median (IQR) within-subject variability in plasma apelin concentration as assessed by testing plasma samples collected between 8:00 a.m. on day −1 to 9:00 a.m. on day 1 was 18.6% (13.3 to 23.6%) and that of plasma AVP concentration was 23.3% (18.2 to 36.8%).

There was no significant difference in plasma apelin or in plasma AVP concentrations between the individuals assigned to the hypertonic saline infusion on day 1 (group 1) and those assigned to the water loading test on day 1 (group 2) at any time point between 8:00 a.m. on day −1 and 9:00 a.m. on day 1 (Table 1). The plasma apelin/AVP ratio was also similar between the two groups (Table 1). There was no significant correlation between plasma apelin concentration and plasma AVP concentration in the absence of osmotic challenge (data not shown). Although plasma osmolality levels significantly differed between the two groups (Table 1), probably because group 1 and group 2 were studied sequentially at different times of the year (see the Concise Methods section), there was no significant correlation between plasma apelin or plasma AVP concentrations and plasma osmolality in the absence of osmotic challenge (data not shown).

Hypertonic Saline Infusion in Group 1

The hypertonic saline infusion induced a smooth and linear increase in plasma osmolality from 288 ± 1.6 mOsm/kg H2O at baseline to 302 ± 3.1 mOsm/kg H2O at the end of the infusion (P < 0.0001 versus baseline; Figure 2A) and in plasma sodium (Na+) concentration (P < 0.0001 versus baseline; Table 2), associated with a parallel linear increase in plasma AVP.

Figure 1. Reverse-phase HPLC analysis of apelin-IR in human plasma. A human plasma sample was chromatographed on a Vydac C4 column, and the immunoreactive material contained in the HPLC fractions was quantified by RIA. This chromatogram is representative of four independent experiments giving the following percentage of each apelin fragment: pE13F, 30 ± 13%; K17F, 56 ± 12%; and apelin 36, 14 ± 7%. The dotted line indicates the acetonitrile gradient. The arrows indicate the elution positions of the reference peptides.
Table 1. Plasma apelin, osmolality, and AVP levels in physiologic unrestricted water intake conditions from day –1 (8:00 a.m.) to day 1 (9:00 a.m.) in 10 healthy male participants.

<table>
<thead>
<tr>
<th>Time</th>
<th>Parameter</th>
<th>Value (Mean ± SD) or Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 a.m.</td>
<td>Plasma apelin (fmol/ml; mean ± SD)</td>
<td>478 ± 167</td>
</tr>
<tr>
<td>12:00 p.m.</td>
<td></td>
<td>443 ± 90</td>
</tr>
<tr>
<td>4:00 p.m.</td>
<td></td>
<td>415 ± 117</td>
</tr>
<tr>
<td>8:00 p.m.</td>
<td></td>
<td>90 ± 4.9</td>
</tr>
<tr>
<td>12:00 a.m.</td>
<td></td>
<td>472 ± 129</td>
</tr>
<tr>
<td>9:00 a.m.</td>
<td></td>
<td>444 ± 46</td>
</tr>
</tbody>
</table>

Plasma apelin/AVP ratio (fmol/fmol; median [IQR])

<table>
<thead>
<tr>
<th>Time</th>
<th>Value (Mean ± SD) or Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 a.m.</td>
<td>233 (188 to 265)</td>
</tr>
<tr>
<td>12:00 p.m.</td>
<td>221 (160 to 230)</td>
</tr>
<tr>
<td>4:00 p.m.</td>
<td>157 (154 to 252)</td>
</tr>
<tr>
<td>8:00 p.m.</td>
<td>231 (144 to 238)</td>
</tr>
<tr>
<td>12:00 a.m.</td>
<td>221 (144 to 238)</td>
</tr>
<tr>
<td>9:00 a.m.</td>
<td>154 (146 to 238)</td>
</tr>
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Plasma osmolality (mOsm/kg H2O; mean ± SD)

<table>
<thead>
<tr>
<th>Time</th>
<th>Value (Mean ± SD) or Median (IQR)</th>
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</thead>
<tbody>
<tr>
<td>8:00 a.m.</td>
<td>285 ± 3</td>
</tr>
<tr>
<td>12:00 p.m.</td>
<td>288 ± 3</td>
</tr>
<tr>
<td>4:00 p.m.</td>
<td>288 ± 3</td>
</tr>
<tr>
<td>8:00 p.m.</td>
<td>288 ± 3</td>
</tr>
<tr>
<td>12:00 a.m.</td>
<td>288 ± 3</td>
</tr>
<tr>
<td>9:00 a.m.</td>
<td>287 ± 3</td>
</tr>
</tbody>
</table>

We then plotted the absolute difference between the observed plasma apelin concentration from 2.6 (2.4 to 3.5) fmol/ml at baseline to 10.0 (8.9 to 11.3) fmol/ml at the end of the infusion ($P < 0.0001$ versus baseline; Figure 2B).

There was a significant and marked decrease in hematocrit ($P = 0.0017$), total protein concentration ($P = 0.0003$), and plasma active renin ($P = 0.0019$) and aldosterone concentrations ($P = 0.0198$) associated with an increase in the estimated extracellular fluid volumes (ECFV; 17.4 ± 1.6%; Table 2). There was a highly significant positive linear correlation between plasma AVP concentration and plasma osmolality between baseline and time 120 min ($r = 0.91, n = 35, P < 0.0001$; Figure 3A), and the extrapolated median osmotic threshold for AVP secretion was 283.1 mOsm/kg H2O (range 278.4 to 285 mOsm/kg H2O).

The time course of plasma apelin concentration was biphasic. During the first 80 min of the hypertonic saline infusion, the plasma apelin concentration decreased linearly from 567 ± 124 fmol/ml at baseline to 326 ± 111 fmol/ml ($P < 0.01$ versus baseline; median change from baseline [IQR] = 43.8% [-47.2 to -20.4%]; Figure 2C), contrary to the plasma AVP concentration. Simple linear regression of plasma apelin and plasma osmolality was thus applied to each individual infusion of hypertonic saline between 0 and 80 min, the linear part of the decrease in plasma apelin with time. There was a highly significant negative linear correlation between plasma apelin concentration and plasma osmolality for the period 0 to 80 min ($r = -0.70, n = 25, P = 0.0004$; Figure 3B). There was also a significant negative linear correlation between plasma apelin concentration and plasma AVP concentration for the period 0 to 80 min ($r = -0.52, n = 25, P = 0.015$; Figure 3C); however, after adjustment for plasma osmolality, the relationship between plasma apelin concentration and plasma AVP concentration was no longer significant ($r = -0.31, n = 25, P = 0.3806$; data not shown). This indicates that the correlation between plasma apelin and AVP concentration observed during the first 80 min of the hypertonic saline infusion was driven by the plasma osmolality.

During the last 40 min of the hypertonic saline infusion, plasma apelin concentration increased from 326 ± 111 fmol/ml at 80 min to 458 ± 136 fmol/ml at 120 min ($P = 0.4961$ versus baseline; Figure 2C). The plasma apelin/AVP ratio decreased massively from 196 (193 to 202) fmol/fmol at baseline to 47 (30 to 50) fmol/fmol at time 80 min ($P < 0.0001$ versus baseline) and remained at a low value up to 120 min (52 [46 to 52] fmol/fmol; $P < 0.0001$ versus baseline; Figure 4).

To search for an explanation for the rise in plasma apelin after its initial decrease, we used the individual equations of the regression line between plasma apelin and plasma osmolality between baseline and time 80 min to predict a curve for plasma apelin between time 0 and time 120 min if plasma osmolality was the sole determinant factor influencing plasma apelin levels. The predicted curve was plotted and compared with the observed curve (Figure 5A). The two curves diverged from time point 80 min to time point 120 min (end of the infusion). We then plotted the absolute difference between the observed
and the predicted apelin values at each time point (Figure 5B).
From 80 min to the end of infusion, the difference between the
observed and the predicted apelin values increased linearly. We
then superimposed the time course of the relative changes in
estimated ECFV from time 0 to time 120 min (Figure 5B). The
observed plasma apelin concentration started to diverge from
the predicted value (i.e., the difference diverged from 0) when
the estimated ECFV had increased by approximately 12 to
15%. The expansion in the estimated ECFV had no apparent
effect on plasma AVP concentrations (see Figure 2B).

Water Loading in Group 2
Plasma osmolality decreased rapidly and significantly from
baseline (284.6 ± 1.8 mOsm/kg H₂O) to a nadir at 60 min
(change from baseline −4.0 mOsm/kg H₂O; 95% confidence
interval −7.0 to −1.0 mOsm/kg H₂O; \( P = 0.0076 \)) then pro-
gressively increased to reach the baseline value at 240 min (Fig-
ure 6A). The plasma Na⁺ concentration decreased simulta-
neously (\( P = 0.0033 \) versus baseline; Table 2). There was a
parallel significant decrease in plasma AVP concentration
from 1.9 (1.8 to 2.1) fmol/ml at baseline to a nadir of 1.5 (1.4 to
1.7) fmol/ml (\( P = 0.0139 \) versus baseline; median variation
from baseline of −30.4% [IQR −45 to −28.6]) followed by a
slight increase by 240 min (Figure 6B). Water loading had no
significant effect on hematocrit or total protein, plasma active
renin, and aldosterone concentrations, indicating no signifi-
cant change in estimated ECFV (Table 2).

In contrast to plasma AVP, the plasma apelin concentration
increased from 335 ± 83 to 527 ± 187 fmol/ml (\( P = 0.0047 \)
versus baseline; median variation from baseline IQR 73.9%
23.4 to 74.5) immediately after water loading (time 30 min;
Figure 6C); it then remained stable at approximately 530
fmol/ml for 210 min (time 240 min). The plasma apelin/AVP
ratio increased massively from 144 (121 to 189) fmol/fmol at
baseline to 424 (222 to 475) fmol/fmol immediately after water
loading (\( P = 0.0007 \) versus baseline; Figure 4); it then re-
mained stable at approximately 300 fmol/fmol for 210
min (time 240 min).

DISCUSSION
The main finding of this study is that acute osmotic stimuli
induce opposite regulation of plasma apelin and AVP concen-
trations in humans, as previously reported in rodents.\(^{10,31}\) Ape-
lin-IR was previously detected in human plasma using com-
mercially available apelin-36 RIA or apelin-12 EIA kits,\(^{32–36}\)
but the data revealed an important disparity in the concentra-

Figure 2. Effects of a 2-h hypertonic saline infusion (0.85 M, 0.06
ml/kg per min) on plasma osmolality (A) and plasma AVP (B) and
apelin (C) concentrations. Plasma osmolality and apelin are ex-
pressed as means ± SEM. Plasma AVP is expressed as median
and IQR. The ANOVA for repeated measurements over time on day
1 with a modeling covariance structure within subjects was signifi-
cant for all three variables. Pair-wise comparisons were tested using
the Dunnett adjustment procedure for multiple tests comparing all
time points with baseline level at 9:00 a.m. on day 1. \(* P < 0.05;\)
\(** P < 0.01; *** P < 0.001\) versus baseline (9:00 a.m. on day 1).
Table 2. Hormonal, electrolyte, hematocrit, and total protein changes during hypertonic saline infusion and water loading.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hypertonic Saline Infusion</th>
<th>Water Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time 0</td>
<td>Time 120</td>
</tr>
<tr>
<td>Plasma sodium (mmol/L; mean ± SD)</td>
<td>138.8 ± 0.9</td>
<td>147.4 ± 0.9</td>
</tr>
<tr>
<td>Hematocrit (%; mean ± SD)</td>
<td>42.9 ± 2.4</td>
<td>38.6 ± 3.0</td>
</tr>
<tr>
<td>Total proteins (g/L; mean ± SD)</td>
<td>69.2 ± 2.7</td>
<td>59.4 ± 2.7</td>
</tr>
</tbody>
</table>
| Plasma active renin (pg/ml; mean ± SD) | 35.8 ± 8.4 | 17.4 ± 3.2 | 22.0 ± 6.4 | 24.0 ± 8.0
| Plasma aldosterone (pg/ml; median [IQR]) | 88 (79 to 145) | 50 (33 to 52) | 114 (84 to 117) | 77 (69 to 120)
| Cumulative Na input (mmol; mean ± SD) | – | 465 ± 36 | – | 0 |
| Cumulative Na output (mmol; mean ± SD) | – | 53 ± 28 | – | 29 ± 13 |
| Cumulative water input (ml; mean ± SD) | – | 550 ± 37 | – | 1546 ± 68 |
| Cumulative water output (ml; mean ± SD) | – | 255 ± 106 | – | 1554 ± 327 |
| ECFV (L; mean ± SD) | 16.0 ± 0.9 | 18.8 ± 1.1 | 16.1 ± 0.4 | 15.9 ± 0.3 |
| ECFV changes (%; mean ± SD) | – | 17.4 ± 1.6 | – | –1.3 ± 0.6 |

*The hypertonic saline infusion caused a significant and marked decrease in hematocrit (P = 0.0017), total protein concentration (P = 0.0003), and plasma active renin (P = 0.0019) and aldosterone (P = 0.0198) concentrations. Water loading had no significant effect on hematocrit or total protein, plasma active renin, and aldosterone concentrations.

**Plasma active renin and aldosterone were measured at time 180.

***Cumulative Na and water input/output are cumulative over 120 min for the hypertonic saline infusion or over 240 min for the water loading test.

The hypertonic saline infusion was followed by a slight increase in plasma apelin concentration during the first 80 min of the hypertonic saline infusion. The initial decrease in plasma apelin concentration during the first 80 min of the hypertonic saline infusion was followed by a slight increase in plasma apelin concentration during the last 40 min. Our data suggest that the rise in the plasma apelin concentration after its initial decrease may be triggered by the infusion-induced increase in ECFV (17.4% above its baseline) as also reflected by the significant and marked decreases in hematocrit, total protein, and plasma active renin, and aldosterone concentrations. In our study, this ECFV expansion had no detectable effect on plasma AVP concentration, although changes in pressure volume are potent nonosmotic stimuli for AVP secretion.

Contrary to the changes in plasma AVP, the linear increase in plasma osmolality had the opposite effect on plasma apelin, the concentration of which declined linearly by 43.8% (20.4 to 47.2%) 30 min after the start of the infusion; during this period, the plasma apelin concentration was highly significantly and negatively correlated to plasma osmolality and to plasma AVP concentration. The initial decrease in plasma apelin concentration during the first 20 min of the hypertonic saline infusion was followed by a slight increase in plasma apelin concentration during the last 40 min. Our data suggest that the rise in the plasma apelin concentration after its initial decrease may be triggered by the infusion-induced increase in ECFV (17.4% above its baseline) as also reflected by the significant and marked decreases in hematocrit, total protein, and plasma active renin, and aldosterone concentrations. In our study, this ECFV expansion had no detectable effect on plasma AVP concentration, although changes in pressure volume are potent nonosmotic stimuli for AVP secretion.

Conversely, water load decreased plasma osmolality and plasma Na⁺ and AVP concentrations. The decrease in plasma osmolality was accompanied by a rapid increase (within 30 min) in plasma apelin concentration to a plateau level. Even though the hypo-osmotic stimulus was modest and not sustained (−4.0 mOsm/kg H₂O; 95% confidence interval −7.0 to −1.0 mOsm/kg H₂O), it was strong enough to maintain the plasma apelin concentration at a plateau for 210 min (73.9% [23.4 to 74.5%]), contrasting with the opposite and modest decrease in plasma AVP concentration (−30.4% [−45 to −28.6%]).
the infusion-induced increase or water load–induced decrease in plasma osmolality both were much larger than the within-subject variability in plasma apelin concentration. This observation is consistent with plasma osmolality being a major physiologic regulator of plasma apelin levels in humans as previously reported in rodents. We previously showed that intracerebroventricular injection of apelin inhibited AVP release in the bloodstream in water-deprived and lactating rodents. Conversely, intracerebroventricular infusion of AVP induced apelin accumulation in magnocellular neurosecretory neurons; this effect is blocked by a V1 receptor antagonist. Moreover, apelin response to dehydration in rats is opposite to that of AVP. Indeed, water deprivation in rats reduces plasma apelin concentrations and induces an intraneuronal pile-up of the peptide in magnocellular AVP neurons, whereas it increases plasma AVP concentrations and decreases AVP neuronal stores because AVP is released faster than it is synthesized. The increased somatodendritic release of AVP during dehydration in rodents optimizes phasic activity of vasopressinergic neurons, facilitating systemic AVP release, whereas concomitant accumulation of apelin in the same neurons (which is thus not systemically and intranuclearly released), prevents the inhibitory action of apelin on AVP neuron activity, thus facilitating systemic AVP release. The opposite regulation of apelin and AVP during water deprivation in rats allows AVP and apelin to act in concert to reinforce the systemic AVP release necessary for a sustained antidiuresis, thus participating in water balance.

Finally, the time course evolution of plasma apelin/AVP ratio with changes in osmolality provided additional information. Indeed, because baseline plasma AVP concentrations...
were approximately 150 to 200 times lower than plasma apelin concentrations on a molar basis, the apelin/AVP ratio revealed more significant differences during both the hypertonic saline infusion (massive decrease from 196 to 48 fmol/fmol) and the water-loading test (massive increase from 144 to 424 fmol/fmol). Thus, the apelin/AVP ratio may indicate the osmolar state in the organism, with low values indicating a tendency toward hyperosmolality and high values a tendency toward hypo-osmolality. Whether the plasma apelin/AVP ratio may be a useful index rather than studying each hormone alone in the diagnosis of hyponatremic/hypo-osmolar states remains to be established in further studies.

Figure 5. (A) Time course of observed (●) and predicted (○) plasma apelin concentration during the hypertonic saline infusion. The equation of the regression line between plasma apelin and plasma osmolality between times 0 and 80 min was then used to predict the curve for plasma apelin between time 80 and time 120 min. (B) Plot of the absolute difference between the observed and the predicted plasma apelin values (left axis, □) and of the relative increase in estimated extracellular fluid volume (right axis, □). The observed and predicted values diverged once estimated ECFV had increased by 12 to 15%. Data are means ± SEM.

Figure 6. Effects of a 20-ml/kg water load within 30 min on plasma osmolality (A) and plasma AVP (B) and apelin (C) concentrations. Plasma osmolality and apelin are expressed as means ± SEM. Plasma AVP is expressed as median and IQR. The ANOVA for repeated measurements over time on day 1 was significant for all three parameters. Then pair-wise comparisons were tested using the Dunnett adjustment procedure for multiple tests comparing all time points with baseline level at 9:00 a.m. on day 1. *P < 0.05; **P < 0.01; ***P < 0.001 versus baseline (9:00 a.m. on day 1).
Our study represents the first demonstration in humans of the regulation of plasma apelin concentration by osmotic stimuli. We also show that this regulation is opposite to that of AVP, consistent with results obtained in animal models after water deprivation.10,31,42 This suggests that apelin, like AVP, may participate in the maintenance of body fluid homeostasis in humans as in rodents. It is still not clear whether the changes in the plasma apelin concentration triggered by changes in osmotic pressure only reflect changes in neurohypophysal secretion or have direct peripheral and/or intrarenal hemodynamic effects15–17 or endocrine effects on water diuresis via binding to intrarenal receptors.12,18 Apelin receptor mRNA has been detected in a subpopulation of cortical glomeruli and in cells along the vasa recta in the inner stripe of the outer medulla, a region of the kidney playing a key role in water and sodium balance.12,18

It would be of interest to investigate plasma apelin levels in parallel with plasma AVP in various pathologic states of euvolemic and hypervolemic hyponatremia. Analyzing the relationship between apelin concentration, AVP concentration, and osmolality in plasma may reveal new classifications of the multiple causes of hypo-osmolar states of impaired urinary dilution or concentration in patients. Finally, the development of nonpeptidic agonists of the apelin receptor could be an alternative or complementary therapeutic approach to V2 receptor antagonists43 for the treatment of water retention and/or hyponatremic disorders.

CONCISE METHODS

Participants
Ten healthy normotensive white male nonsmoker volunteers aged 18 to 25 yr (body mass index 23.5 ± 1.9 kg/m²) completed the study after giving written and informed consent. The protocol was approved by the ethics committee “CCPPRB, Paris-Cochin, France,” and the investigation was conducted according to Declaration of Helsinki principles.

Study Protocol
Participants were instructed to arrive at the Clinical Investigation Center at 7:00 a.m. on day 1 and remained hospitalized for 36 h. Fluid intake throughout day 1 was unrestricted until 9:00 p.m., and participants were given light meals at 9:30 a.m., 12:00 p.m., and 8:00 p.m. (NaCl approximately 150 mmol/d). From 9:00 p.m. until 9:00 a.m. on day 1, participants refrained from water and food intake. At 9:00 a.m. on day 1, after a 12-h overnight fast and water restriction, five participants received an infusion of a 2-h 0.85-M saline solution (5%) at the rate of 0.06 ml/kg per min to increase plasma osmolality (group 1). The five remaining participants ingested 20 ml/kg body wt water within 30 min to decrease plasma osmolality (group 2). In the two groups, blood was sampled at various times on days 1 and 1 for plasma osmolality, Na⁺, apelin, AVP, active renin, aldosterone, hematocrit, and total protein determinations with the participants being semirecumbent for 1 h before each blood sampling. Urine was collected before and during the tests for determination of electrolytes.

The two experiments (group 1 and group 2) were independent and performed sequentially at two different periods of the year (autumn to winter for group 1 and late spring to summer for group 2). Each group was its own control.

Laboratory Methods
Plasma osmolality was measured by cryoscopy (Fiske Mark3 osmometer, Norwood, MA). The methods used for collecting blood samples and for quantifying plasma AVP, active renin (immunoradiometric assay), and aldosterone (RIA) were as described previously.44,45 For plasma apelin measurements, blood was sampled into prechilled EDTA-K₃ tubes and centrifuged at 1600 × g at 4°C for 15 min and stored at −80°C.

For HPLC analysis, human plasma samples were prepurified on a Sep-Pak C₁₈ cartridge (Waters Associates, Milford, MA). Bound material was eluted with acetonitrile/50%, and the eluate was evaporated under reduced pressure. The extract was analyzed by reversed-phase HPLC on a 0.46 × 25-cm Vydac 214TP54 C4 column equilibrated with a solution of acetonitrile/water/TFA (10.5:89.4:0.1; vol/vol/vol) at a flow rate of 1 ml/min. The concentration of acetonitrile in the eluting solvent was raised to 24.5% over 90 min. One-minute fractions were collected and assayed for apelin-IR. Synthetic pE13F, K17F, and apelin 36 (2 μg each), used as reference peptides, were subjected to chromatography under the same conditions as human plasma samples.

For apelin RIA, plasma samples (0.75 ml) were mixed with 0.05 ml of 1% BSA and 0.25 ml of 3 M HCl and centrifuged at 1600 × g at 4°C for 10 min. The supernatants were collected and adjusted to pH 6.5 with 12 M NaOH and 2 M Tris-HCl buffer (pH 7.4). The samples were then mixed with 1 ml of 1% trifluoroacetic acid (TFA) supplemented with 0.05% BSA and loaded onto a Sep-Pak C₁₈ cartridge (Waters Associates) equilibrated with 5 ml of 1% TFA. The columns were washed with 3 ml of 1% TFA, and apelin peptides were eluted with 1.5 ml of 100% acetonitrile. The recovery (mean ± SEM) was 91 ± 3%. The eluates were dried and dissolved in 0.55 ml of RIA buffer.

Immunoreactive apelin concentration was measured by RIA using highly selective rabbit polyclonal antibodies directed against the apelin fragment K17F produced in the laboratory.10 This antibody recognizes two epitopes in K17F, one of them being common to pE13F and K17F. We thus used iodinated pE13F as a tracer to make sure to measure equivalently K17F and pE13F endogenous levels. When using iodinated pE13F as a tracer and defining reactivity with pE13F as 100%, our antibodies cross-react 214% with K17F, 100% with pE13F, and 100% with apelin 36. Reactivity decreased with loss of amino acids from the N-terminal part of K17F and was negligible with C-terminally truncated fragments of K17F and various other bioactive peptides, including angiotensin II, angiotensin III, neuropeptide Y, and AVP. Plasma samples (0.1 ml) were mixed with [¹²⁵I]pE13F (15,000 cpm) and K17F antiserum (1:10,000) to give a total volume of 0.2 ml and incubated at 4°C overnight. Then, the antigen–antibody complex was immunoprecipitated and quantified as described previously.10 The detection limit was 6 fmol/ml, and the quantification limit was 12 fmol/ml. The within- and between-assay coefficients of variation were 3 and 5%, respectively. Serial dilutions of human plasma samples gradually inhibited binding of [¹²⁵I]pE13F to the an-
tserum, and inhibition curves paralleled that of pE13F used as a standard (data not shown).

Statistical Analyses

Data are expressed as medians with IQR for variables known to be non-normally distributed and for all relative variations from baseline. For all other measurements, data are expressed as means ± SD. Data were analyzed using ANOVA for repeated measurements over time with a modeling covariance structure within subjects. When the global time effect was significant, pair-wise comparisons were tested using the Dunnett adjustment procedure for multiple tests. Correlations between variables were estimated using analysis of covariance after withdrawal of the subject effect. P < 0.05 was considered to be significant. SAS statistical software version 8.2 (Cary, NC) was used for statistical analyses.

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DISCLOSURES

None.

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