Atrial natriuretic peptide inhibits fluid intake in hyperosmolar subjects

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1. The effect of atrial natriuretic peptide on osmotically stimulated thirst appreciation and consequent fluid intake was investigated in healthy man.
2. Six seated male subjects were studied on two occasions: synthetic α-human atrial natriuretic peptide (99–126) (2 pmol min⁻¹ kg⁻¹) or placebo (saline, 150 mmol/l NaCl) was infused intravenously for 105 min; 30 min after the start of atrial natriuretic peptide/placebo infusion, hypertonic saline (855 mmol/l NaCl) was infused (0.06 ml min⁻¹ kg⁻¹) for 60 min. Subjects were then allowed free access to water for the next 2 h; infusion of atrial natriuretic peptide/placebo continued for the first 15 min of the drinking period.
3. The plasma atrial natriuretic peptide concentration did not alter significantly during infusion of hypertonic saline and placebo; it rose to a steady state of 12.7 ± 1.1 pmol/l (mean ± SEM) during the infusion of atrial natriuretic peptide and hypertonic saline, and remained at this level during the first 15 min of the drinking period. During infusion of hypertonic saline and atrial natriuretic peptide or placebo, similar increases in plasma osmolality (P < 0.001) and plasma vasopressin concentration (P < 0.005) occurred. During infusion of hypertonic saline and atrial natriuretic peptide or placebo, thirst increased significantly over the time course of both studies (P < 0.01), but the effect of atrial natriuretic peptide infusion compared with placebo infusion was to significantly decrease thirst at 60 min.
4. Drinking rapidly abolished thirst and vasopressin secretion before changes in plasma osmolality occurred. Subjects drank significantly less water after atrial natriuretic peptide infusion compared with after placebo infusion (P < 0.01).
5. In conclusion, physiological increases in plasma atrial natriuretic peptide concentrations blunt osmotically stimulated thirst appreciation and attenuate subsequent fluid intake in hyperosmolar man.

INTRODUCTION

Although the body has several intrinsic mechanisms to minimize water loss, thirst, the physiological drive to drink water, is essential to maintain fluid balance. Animal studies have demonstrated that the peripheral natriuretic and diuretic actions of atrial natriuretic peptide (ANP) [1] are matched by a central action to inhibit salt appetite [2] and water intake [3–9]; it certainly makes teleological sense that a hormone which causes a diuresis should also inhibit fluid intake. To date, there have been few studies in man which have related thirst, drinking behaviour and circulating ANP concentration. Thirst is normally a continuous variable, rising when subjects are rendered increasingly hyperosmolar [10]. However, we have previously shown that peripherally infused ANP blunts osmotically stimulated thirst appreciation in man [11]. The aim of this study was to assess the effect of physiological increases in circulating ANP concentrations on actual fluid intake in hyperosmolar man. The results of this study have been published in abstract form [12].

METHODS

Subjects

All subjects gave their informed consent to the studies, which had the approval of the local Ethical Committee. Six healthy male volunteers were studied on two occasions in random order separated by at least 1 month. They were aged 27 ± 1 years (mean ± SEM) and were not taking any medication. Subjects abstained from alcohol and nicotine for 24 h before the study; they fasted, but had free access to tap water from 24.00 hours on the evening before the study.

Protocol

On the morning of the study, subjects were weighed, passed urine and rested for 30 min in the

Key words: atrial natriuretic peptide, fluid intake, thirst.

Abbreviations: ANP, atrial natriuretic peptide; HS, hypertonic saline (855 mmol/l NaCl); VAS, visual analogue scale.

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seated position before the study began. Studies were performed seated, as in this position the release of ANP is not stimulated by hypertonic saline (HS) infusion [13]. Subjects were advised that they would receive an infusion of HS which might cause an increase in thirst [14]; they were also told they would receive either ANP or a placebo infusion, although they would not know which until after both studies had been performed.

The visual analogue scale (VAS) for recording thirst comprised a 10 cm uncalibrated line [10]. The subjects were asked 'how thirsty do you feel?' and told to mark the line at the point representative of their thirst between the extremes of very severe thirst at 10 cm and no thirst at 0 cm. At regular times during the study, this process was repeated on a separate line, but no reference was made to previous ratings. At each sampling time, the subjects were asked about possible symptoms of nausea and in addition to their thirst appreciation. Two intravenous cannulae were inserted into the antecubital veins of one forearm, one for infusion of HS (855 mmol/l NaCl) and another for infusion of ANP/placebo; a third cannula was inserted into the opposite forearm for blood sampling. Blood pressure and pulse were recorded at 15 min intervals using an automatic sphygmomanometer (Accutorr, Cambridge, U.K.) attached over the brachial artery of the arm used for blood sampling. The amount of fluid consumed during the drinking period was assessed by using a measuring jug.

Sampling and analysis

A sample of blood was withdrawn at time -30 min and an infusion of synthetic α-human ANP (99-126) (Shire Pharmaceuticals, Andover, Hants, U.K.) in 60 ml of vehicle (150 mmol/l NaCl) (at 2 pmol/min⁻¹ kg⁻¹) or placebo (saline, 150 mmol/l) was started at a rate of 0.5 ml/min and was continued for 105 min.

At time zero (i.e. 30 min after starting the ANP/placebo infusion), an infusion of HS (855 mmol/l NaCl) was begun at a rate of 0.6 ml min⁻¹ kg⁻¹ and was continued for 1 h. Subjects were then allowed free access to tap water for the next 2 h; infusion of ANP or placebo continued for the first 15 min of this 2 h period.

Blood was sampled at 15 min intervals for the first 90 min and at +5, +10, +15, +30, +60 and +120 min during the drinking period. Venous blood was withdrawn into cooled syringes and was transferred to chilled EDTA (potassium salt)/Trasylol (1000 kallikrein inhibitory units) tubes (4 ml) and lithium heparin tubes (5 ml). Aliquots of blood were taken from the lithium heparin tubes into heparinized capillary tubes for duplicate measurement of packed cell volume (Hawkesley microhaematocrit centrifuge) and the remaining blood was centrifuged at 4°C for 20 min (2000 g). Within 5 min of sampling, the plasma was separated and divided into aliquots for measurement of serum sodium concentration (ion-exchange electrode, Astra-8, Beckman), plasma osmolality (depression of freezing point method automatic micro-osmometer; Roebling) and plasma vasopressin concentration using a previously described r.i.a. (inter- and intra-assay coefficients of variation 12.0% and 4.3% respectively; limit of detection 0.3 pmol of vasopressin/litre of plasma) [15]. Blood taken into EDTA (potassium salt)/Trasylol tubes was spun, separated and the plasma was stored at -80°C until measurement of plasma ANP concentration within 1 week by a sensitive and specific r.i.a. (inter- and intra-assay coefficients of variation 13.1% and 10.1%, respectively; limit of detection 1.2 pmol of ANP/litre of plasma) [16].

Statistics

Results are expressed as means ± SEM. For statistical purposes, the difference in thirst rating was calculated as the distance (in cm) of the subjects' mark from initial thirst at -30 min [17]. Undetectable ANP concentrations were given a value of half the detection limit (i.e. 0.6 pmol/l). Mean arterial blood pressure was calculated by adding one-third of the pulse pressure to the diastolic pressure. The change in blood volume was calculated using standard formulae [18].

Two-way analysis of variance was performed on the data, and the significance of individual effects (i.e. time and treatment) was further analysed using Duncan's range test.

RESULTS

Both studies were well tolerated by all subjects and there were no side-effects associated with either study.

Fig. 1 illustrates the changes in serum sodium concentration and plasma osmolality over the course of the studies. Serum sodium concentration rose from 139.7 ± 0.2 to 145.0 ± 0.9 mmol/l during infusion of placebo plus HS (P < 0.001) and from 138.8 ± 0.5 to 144.8 ± 1.2 mmol/l during infusion of ANP plus HS (P < 0.001); during the 2 h drinking period, serum sodium concentration fell to 141.5 ± 0.9 mmol/l during the placebo study (P < 0.05) and to 140.5 ± 0.3 mmol/l during the ANP study (P < 0.05). Infusion of HS caused a progressive linear increase in plasma osmolality, from 286.5 ± 0.8 to 295.5 ± 1.2 mosmol/kg during placebo plus HS infusion (P < 0.001) and from 285.6 ± 1.4 to 295.8 ± 1.2 mosmol/kg during ANP plus HS infusion (P < 0.001); in the 2 h drinking period, plasma osmolality fell to 288.4 ± 1.0 mosmol/kg during the
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ANP/placebo infusion

HS infusion

Drinking period

placebo study ($P<0.001$) and to $289.3 \pm 1.8$ mosmol/kg during the ANP study ($P<0.001$). The plasma vasopressin concentration rose from $1.2 \pm 0.3$ to $2.8 \pm 0.8$ pmol/l during placebo plus HS infusion ($P<0.005$) and from $0.9 \pm 0.2$ to $2.6 \pm 0.9$ pmol/l during ANP plus HS infusion ($P<0.005$). During the 2h drinking period, plasma vasopressin concentrations fell to $0.6 \pm 0.3$ mmol/l during the placebo study ($P<0.001$) and to $0.8 \pm 0.5$ mmol/l during the ANP study ($P<0.001$). There was no significant effect of ANP on plasma osmolality, serum sodium concentration or plasma vasopressin concentration compared with placebo.

Fig. 2 illustrates the change in plasma ANP concentration, the change in the subjective rating of thirst over the course of the studies and the amount of fluid drunk during the drinking period. The plasma ANP concentration did not alter during the placebo plus HS study, but rose to a steady state of $12.7 \pm 1.1$ pmol/l during the ANP plus HS study. During the 2h drinking period, the plasma ANP concentration did not change in the placebo study, but in the ANP study fell rapidly to basal levels once the ANP infusion was stopped. The plasma ANP concentration was significantly different from that in the control study at times $-15, 0, 15, 30, 60, +5, +10$ and $+15$ min ($P<0.05$ or $P<0.01$: see Fig. 2 for individual values).

The change in thirst was determined from the difference on the VAS from the value for each individual at initial thirst at $-30$ min. Thirst varied as an effect of both time and ANP/placebo infusion, and at $60$ min ANP significantly depressed the thirst rating (placebo plus HS $4.3 \pm 0.9$ cm; ANP plus HS $1.8 \pm 1.1$ cm; $P<0.05$). There was a rapid fall in thirst appreciation during the drinking period in both studies ($P<0.001$). The higher plasma ANP concentrations during the first 15 min of drinking in the ANP study did not effect the fall in thirst.
appreciation, which varied only as a function of time.

The total amounts of water drunk at individual
time points during the two studies are shown in Fig.
2; by 5 min, subjects had drunk 41% (placebo study)
and 33% (ANP study) of the total volume. At
15 min, 60% of the total volume had been drunk in
both studies. The amount of fluid drunk varied as
an effect of both time and ANP/placebo infusion
and at 30 min subjects had drunk significantly more
after the placebo infusion compared with after the
ANP infusion (30 min: placebo plus HS
928±102 ml; ANP plus HS 504±145 ml, P<0.05);
at 60 min, the amounts drunk were 1095±134 ml
during placebo study and 595±119 ml during the
ANP study (P<0.01). By the end of the drinking
period, subjects had drunk 1261±208 ml in the
placebo study, and 683±110 ml in the ANP study
(P<0.01).

Packed cell volume fell from 45.2±0.8% to
41.5±0.8% (P<0.05) during placebo plus HS
infusion and from 45.2±1.2 to 42.6±1.1% during
ANP plus HS infusion (not significant), which represen-
tes increases in blood volume of 7.9±2.5% and
5.7±1.6%, respectively. Packed cell volume varied
as a result of time and not treatment in the placebo
study. During the drinking period, packed cell
volume rose to 43.5±0.8% in the placebo study (not
significant) and to 43.0±1.5% in the ANP study
(not significant). Mean arterial blood pressure did
not alter significantly over the time course of the
two studies during either infusion of HS or the
drinking period.

DISCUSSION

Human studies investigating the relationship be-
between ANP, thirst and fluid intake are limited. We
have previously used a VAS to provide a semi-
quantitative estimation of thirst, and demonstrated
that ANP inhibits osmotically stimulated thirst in
man [11]. Owing to the study design, it was not
possible to correlate the thirst estimate with fluid
intake; in the present study, we have verified our
estimates of thirst by assessing the amount of water
drunk spontaneously after the hyperosmolar stimu-
lus. The results show that a physiological increase in
plasma ANP concentrations attenuates fluid intake
in normal male subjects. We have also confirmed
that peripherally infused ANP has no effect on
osmotically stimulated vasopressin secretion [11],
and that the release of ANP is not stimulated by
drinking [19].

Several aspects of the results merit further dis-
cussion. Despite a fall in plasma ANP concen-
trations and thirst ratings to basal values during
the drinking period, the difference in fluid intake in
ANP-infused, compared with placebo-infused, sub-
jects persisted. It is known that the biological effects
of ANP are prolonged compared with the plasma
half-life, and one can speculate that ANP, remaining
bound to its receptors after clearance from the
plasma, continued to exert an inhibitory effect on
fluid intake.

Similarly, despite a significantly lower cumulative
fluid intake in ANP-infused subjects, there was no
significant difference between the two studies in the
final plasma osmolality or serum sodium concen-
tration attained. This finding could be explained
in terms of a persistent natriuretic action of ANP,
by direct effects on glomerular filtration rate and
segmental tubular transport [20]. This would lead
to an enhanced natriuresis during the drinking
period after the ANP infusion compared with the
drinking period after the placebo infusion. Un-
fortunately, urinary sodium excretion was not mea-
sured during either study.

Blood volume increased during both studies, but
this increase reached significance only during pla-
cebo plus HS infusion. The difference between the
two studies is likely to be due to ANP-induced
shifts in fluid from the intravascular compartment
to the extravascular space possibly mediated by the
action of ANP in increasing capillary permeability.

We have reported on the antidipsogenic effect of
peripherally infused ANP on osmotically stimulated
fluid intake in man, and similar results have been
obtained after osmotic stimulation of thirst in the
pig [9]. Studies in the rat, rabbit and pig have
described the effect of peripherally or centrally
infused ANP on extracellular thirst (haemorrhage,
angiotensin II, peritoneal dialysis); all studies
showed inhibition of drinking [3, 4, 6, 8, 9]. Central
and peripheral administration of ANP will also
inhibit fluid intake in response to water deprivation
(mixed cellular and extracellular stimulus) in the rat
[3, 4, 5] and goat [7], but not the rabbit [8].

As both intravenous and central infusions of ANP
inhibit drinking, the source and the site of action
of endogenous atrial peptides involved in water intake
are unclear. It was initially argued that any ANP
detected in the central nervous system merely re-
presented sequestration from plasma, but under
normal circumstances ANP does not cross the
blood–brain barrier [21]. A simple experiment using
a r.i.a. indicated at least partial autonomy of brain
ANP from that in the heart. After 3 days of water
depreivation in the rat, there was a reduction in
circulating ANP levels, an increase in atrial ANP
content and a reduction in ANP-like immuno-
reactivity in areas both inside (suprachiasmatic nuc-
elus, supraopticot nucleus) and outside (organum vascu-
losum of the lamina terminalis, neural lobe) the
blood–brain barrier [22].

Several groups [23–26] have reported ANP-
positive neurons, fibres and binding sites in areas of
the brain associated with the control of fluid and
electrolyte balance, such as the hypothalamus and
the circumventricular organs [27]. The presence of
ANP mRNA within the brain indicates local synthe-
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