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Relationship between thirst perception and plasma arginine vasopressin concentration in man

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Summary: We examined the possibility that measurements of thirst perception in man using the visual Analogue Scale (VAS) can be used to estimate plasma arginine vasopressin concentration in man. In thirty normal subjects (male=15 and female=15), thirst perception (TP, cm) was rated and 5.0ml blood samples were collected for the measurement of plasma arginine vasopressin (P_{AVP}) using Enzyme Immunoassay kit. Male subjects were statistically significantly older and taller than the females. However, the blood pressures, body weight and body mass index were similar. There was no significant difference, male vs. female in TP (5.26±0.51 vs. 5.39±0.53cm), calculated plasma osmolality from TP, P_{osm} (298.5±1.7 vs. 299.0±1.8mOsm/kgH_2O) and measured plasma arginine vasopressin, P_{AVP} (4.85±0.30 vs. 4.71±0.31pg/ml). Furthermore, the calculated P_{AVP} from TP, P_{AVP}-TP was similar (5.40±0.69 vs.5.60 ±0.70pg/ml). When P_{AVP} was calculated from plasma osmolality, P_{AVP}-P_{osm} the values were also similar (6.10±0.70 vs. 6.30±0.80pg/ml). There was no statistically significant difference between the measured P_{AVP} as well as those calculated from TP and from plasma osmolality. It is thus reasonable to conclude that plasma arginine vasopressin concentration maybe estimated using thirst perception and/or plasma osmolality.

Keywords*:* Thirst perception, Arginine vasopressin, Plasma osmolality

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INTRODUCTION

Arginine Vasopressin (AVP) is a 9 amino acid peptide with a 6-member disulfide ring, H-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2. It is structurally related to oxytocin but differing in 2 amino acids. It is synthesized in the supraoptic and paraventricular nuclei of the hypothalamus, and stored in the posterior pituitary. AVP has powerful antidiuretic action and it is therefore also known as antidiuretic hormone (ADH) (Tijssen, 1985). It acts on the collecting tubule of the kidney increasing permeability to water and urea. It also has neurotransmitter and peripheral humoral functions.

AVP has been shown to be released by both osmotic and non-osmotic stimuli (Clarke et. al., 1979; Malvin, 1971), and its release into peripheral blood causes effects on a number of factors, including emotional stress, posture, blood volume, and temperature (Lester and Nelson, 1981, Schrier and Goldberg, 1980). Alcohol inhibits AVP secretion.
Serum AVP measurement is used clinically for studies involving diabetes insipidus, syndrome of inappropriate ADH secretion (SIADH), ectopic AVP production and psychogenic water intoxication (Haynes, 1958).

In healthy adults, who have no fluid restrictions and have a normal activity level, the normal range of plasma concentration of ADH is between 0.35 and 1.94 ng/l (0.32 - 1.80 pmol/l) (Kamath, 2010). Karkare, (2010) showed that if serum osmolality is more than 290mOsm/kg H_2O, the ADH levels should be around 2 – 12 pg/ml, while if serum osmolality is less than 290mOsm/kg H_2O, the ADH levels should be less than 2 pg/ml.

Robertson (1984), defined thirst as "a generalized deep-seated feeling of a desire for water". Thus, thirst is not synonymous with drinking, because drinking can be affected positively or negatively by a variety of factors such as personal and cultural factors. Oral fluid loads and dehydration show a consistent thirst perception in man (Obika et. al., 2009). The stimuli for thirst include, increase in plasma osmolality, decrease in blood volume, decrease in blood pressure, increase in Angiotensin II concentration and dryness of mouth (Guyton and Hall, 2005).

Attempts have been made to assess and relate thirst perception and plasma arginine vasopressin. In 1977, Thompson and Campbell devised a rating scale to quantify acute changes in thirst in controlled experimental settings. Using this method, Robertson (1984), assessed the effects of osmotic stimuli on thirst mechanism and vasopressin secretion at various times during an infusion of hypertonic NaCl solution in healthy adults. The result showed the function: P_{AVP} = 1.48 (Posm - 284.7), and thirst perception, TP, cm = 9.06 (P_{osm} - 293.5). According to his analysis, the osmotic threshold for the onset of thirst was 293.5mOsm/Kg, which is approximately 10mOsm/Kg above the osmotic threshold of vasopressin release.

Thompson et al., (1986) employing the visual analogue scale (VAS), where subjects defined their own thirst ratings before the experiment rather than be assigned to zero thirst ratings as shown by Rolls et. al., (1980), explored the characteristics of osmotically induced thirst throughout a wider range of plasma osmolalities than previously examined. From linear regression of their results, they defined the functions, Thirst Perception (TP, cm) = 0.3 (P_{osm} - 281); and P_{AVP} (pmol/l) = 0.43 (P_{osm} - 284.3). They concluded that (1) the osmolar threshold for onset of thirst (281mOsm/Kg) is at the lower end of the physiological range of plasma osmolality and was lower than that quoted in previous studies (Robertson et. al., 1976; Baylis & Robertson, 1980; Robertson, 1984); (2) thirst perception (TP) rises in a progressive fashion throughout a wide range of plasma osmolalities and that the osmolar threshold for thirst onset of thirst (281mOsm/Kg) was similar to the theoretical osmolar threshold for vasopressin release (285mOsm/Kg).

In the works of Robertson (1984) and Thompson et. al., (1986) it was not clear whether a linear relationship also exist between Thirst Perception and P_{AVP}. However, using the equations of Thompson et. al., (1986):

$$P_{osm} = \frac{10TP}{3} + 281 \quad \text{and} \quad \text{.....(1)}$$

$$P_{osm} = \frac{P_{AVP}}{0.43} + 284.3 \quad \text{.....(2)}$$

Igbokwe and Obika (2008), in their study established the equation:

$$TP_{(cm)} = 0.75\, P_{AVP} + 1.2 \quad \text{…….(3)}$$

Therefore,

$$P_{AVP} = \frac{TP_{(cm)} - 1.2}{0.75} \quad \text{…….(4)}$$

From the equation, it was stated that the lower set point of "not thirsty" in the VAS has a mean TP value of 1.2 cm and a corresponding 285mOsm/Kg H_20 of plasma osmolality. They concluded that the threshold for thirst perception using the VAS lies between thirst perception of 1.50 and 1.58 cm marking on the VAS, plasma osmolality of between 286.0 and 286.3mOsm/Kg H_20 and a corresponding plasma arginine vasopressin concentration of between 0.4 and 0.5 pmol/l. These therefore can be equated to being the sensitivity ranges of the osmoregulatory unit. Markings of between 0.0 and 1.4 cm on the VAS, and plasma osmolality of between 281 and 285mOsm/Kg H_20 are hence normal ranges of "not thirsty".

It is important to validate the equation (4) put forward by Igbokwe and Obika (2008) and ascertain the relationship between plasma arginine vasopressin concentration and thirst perception ratings. The visual analogue scale is an indirect approximation of the plasma osmolality (Robertson et al., 1982). Furthermore, this study compared the differences between the measured and calculated plasma arginine vasopressin concentrations in man with the hope of using TP ratings to calculate an estimate of plasma arginine vasopressin concentration.

MATERIALS AND METHODS

Subjects

Thirty normal subjects (males = 15 and females = 15) between the ages of 18 – 30 years were used in this study. All the subjects were active but none was athletically trained as defined by the absence of a regular physical exercise programme during the last six months before the experiment (Kokkinos et. al., 1995). Exclusion criteria for this study were any history of diabetes and cardio-respiratory disease.

Procedure

Each subject reported at the laboratory on the day of the experiment. Participants were adequately informed of the experimental procedures and they all consented to it. The subject's anthropometric data were then obtained. The height (HT, m) and weight (WT, Kg) were measured using a meter rule and weighing scale respectively, while the body mass index (BMI) was calculated from:

$$BMI = \frac{WT\ (kg)}{HT\ (m^2)}$$

Base-line (resting) blood pressure (BP) was measured with the subject in the seated position and after fifteen minutes rest in the laboratory at a room temperature of 29^0C. The right arm supported at the heart level was used for the measurement. Measurements were made with the aid of the stethoscope and sphygmomanometer. Three readings were obtained on each subject at 3 minutes interval

and the mean of these readings recorded as the normal blood pressure.

Thirst Perception Rating

Thirst perception rating (TP, cm) was obtained using the Visual Analogue Scale (VAS) (Thompson et. al., 1991; Takamata et. al., 1994). There was a separate sheet of paper for each subject with a 10cm marking, the ends of which were marked "very thirsty" and "not thirsty".

Subjects were first presented with instructions for completing the VAS. They then rated how thirsty they were by a mark across each scale.

Plasma Osmolality

The plasma osmolality was obtained indirectly from thirst perception using the equation:

$$P_{osm} = \frac{10TP}{3} + 281 \text{ (Thompson et. al., 1986)}.....(1).$$

The visual analogue scale, an index of TP, is an indirect approximation of the plasma osmolality (Robertson et. al., 1982).

Plasma arginine vasopressin (P_{AVP}) concentration measurement.

Test Principle:

Assay Designs' Vasopressin Enzyme Immunoassay (EIA) kit is a competitive immunoassay for the quantitative determination of vasopressin in samples. The kit uses a polyclonal antibody to vasopressin to bind, in a competitive manner, the vasopresin in the standards or samples or an alkaline phosphatase molecule which has vasopressin covalently attached to it. After a simultaneous incubation at 4^0C for 18 - 24 hours, the excess reagents were washed away and substrate was added. After incubation for 1 hour, the enzyme reaction is stopped and the yellow colour generated read on a microplate reader at 405nm. The intensity of the yellow colour is inversely proportional to the concentration of vasopressin in either standards or samples (Chard, 1990; Tijssen, 1985). Similar procedures have been employed in previous studies by Engvall and Perlman (1971), Edwards (1971), Uno et. al., (1982), Zasshi (1989a & b), Proux et. al., (1993), Sachidhanandam et. al., (2010) and Cayman Chemical Company (2011).

Blood Sample Collection and Handling

Blood samples were drawn from the ante-cubital vein of each subject into chilled EDTA specimen bottles. The samples were centrifuged at 1,600 x g for 15 minutes at 4^0C. The plasma was transferred to a plastic tube and stored at -70^0C.

Assay Precautions:

All reagents were allowed to warm to room temperature for at least 30 minutes before opening

The pipet tip was pre-rinsed with reagent. Fresh pipet tips were used for each sample. Standards and samples were pipetted to the bottom of the wells. The reagents were added to the side of the well to avoid contamination. Unused wells were kept desiccated at 4^0C in the sealed bag provided. Care was taken to ensure that there was no residual wash buffer in the wells as any remaining wash buffer may cause variation in the assay results.

Reagent Preparation:

1. Vasopressin Standard

-The 10,000pg/ml vasopressin standard solution was allowed to warm to room temperature.

-Seven 12 x 75mm glass tubes were labelled No. 1 to No. 7.

-900 µl of standard diluent (Assay Buffer) was pipetted into tubes No 1 (and diluted serially) as follows.

-600 µl of standard diluent was pipetted into tubes No 2, 3, 4, 5, 6 and 7.

-100 µl of the 10,000pg/ml standard solution was added to tube No 1. It was vortexed thoroughly.

-400 µl of tube No 1 was added to tube No 2 and vortexed thoroughly.

This was done for tubes No 3, 4, 5, 6 and 7. Therefore, the concentration of vasopressin in tubes No 1 through No 7 was 1000, 400, 160, 64, 25.6, 10.24, and 4.10 pg/ml respectively. Diluted standards were used within 60 minutes of preparation.

2. Vasopressin conjugate preparation was allowed to warm to room temperature.
3. Wash buffer was prepared by diluting 5ml of the supplied concentrate with 95 ml of deionised water.

Assay Procedure:

All standards and samples were run in duplicate.

-100 µl of the standard was pipetted into the appropriate wells.

-100 µl of the samples was pipetted into the appropriate wells.

-50 µl of the blue conjugate was pipetted into each well except the Total Activity (TA) and Blank wells

-50 µl of the yellow Antibody was pipetted into each well except the Total Activity (TA) and Blank wells.

-The plate was tapped gently to mix. The plate was then sealed and incubated at 4^0C for 18-24 hours.

-The contents of the plate were emptied and washed by adding 400 µl of wash solution to every well.

-The washing was repeated for 2 more times making a total of 3 washes.

-After the final wash, the wells were emptied and the plate was tapped dry on a lint free paper towel.

-200 µl of the p-nitrophenyl phosphate substrate solution was added to every well and incubated at 37^0C for 1 hour without shaking.

-50 µl of stop solution was added to every well. This stopped the reaction and the plate was read immediately. -The plate reader was calibrated and standardized and the standards, controls and samples were read automatically using the logit-log paper plot, where per cent bound was plotted against concentration of vasopressin for the standards. A straight line was approximated through the points and the concentration of vasopressin in the samples determined by interpolation.

Plasma arginine vasopressin concentration was also calculated using thirst perception (TP) and plasma osmolality (P_{osm}) using the following equations:

$$P_{AVP} = \frac{TP_{(cm)} - 1.2}{0.75}$$ (Igbokwe and Obika, 2008)….(2).

And

P_{AVP} = 0.43 (P_{osm} – 284.3) (Thompson et. al., 1986) ……..... (3).

Statistical Analysis

Data are presented as means ± SEM. Differences in TP, P_{AVP}, plasma osmolality (P_{osm}), WT, HT, BMI, age and BP between the groups were analysed using the unpaired students' t-test. Differences in the measured P_{AVP} and P_{AVP} calculated from TP and from plasma osmolality among the groups were analyzed using the one way analysis of variance (ANOVA). Changes were considered statistically significant when $P \leq 0.05$.

RESULTS

The anthropometric data of the subjects are presented in table 1. Male subjects were statistically significantly older and taller than the females. However, the blood pressures, body weight and BMI were similar.

As shown in Table 2 and Figure 1, there was no statistically significant difference, male vs. female in TP (5.26 ± 0.51 vs. 5.39 ± 0.53 cm), calculated plasma osmolality from TP, (298.5 ± 1.7 vs. 299.0 ± 1.8 mOsm/kgH_2O), and measured plasma arginine vasopressin (4.85 ± 0.30 vs. 4.71 ± 0.31 pg/ml).

Table 1.
Anthropometric data of the subjects, expressed as mean ± SEM.

	MALE (n = 15)	FEMALE (n = 15)
Age, yr.	27.4 ± 0.62	24.7 ± 0.69*
Weight, Kg.	72.6 ± 1.75	68.7 ± 2.43
Height, m.	1.72 ± 0.01	1.64 ± 0.01*
BMI, Kg/m^2	24.5 ± 0.52	25.5 ± 0.76
SBP, mHg.	119 ± 1.73	115 ± 1.95
DBP, mHg.	78 ± 1.25	74 ± 1.82

BMI, Body Mass Index; SBP, Systolic blood pressure; DBP, Diastolic Blood Pressure; * P < *0.05*

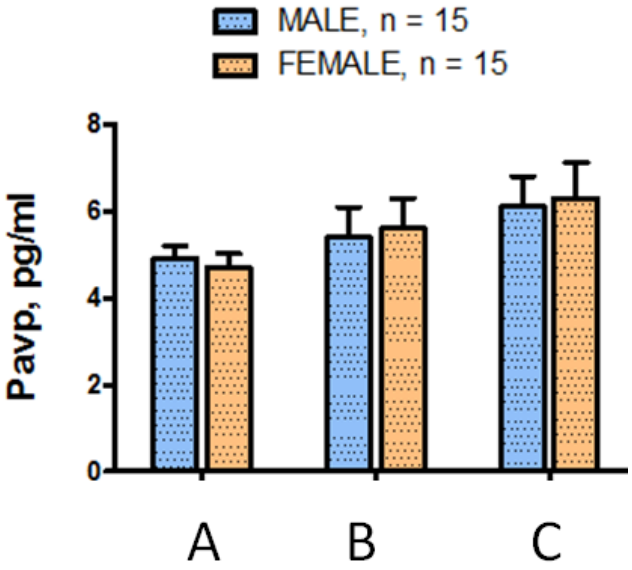

Figure 1.
Plasma arginine vasopressin in normal male and female subjects, Data shown are Mean ± SEM of measured (A) and calculated P_{AVP} from TP (B) and from plasma osmolality (C).

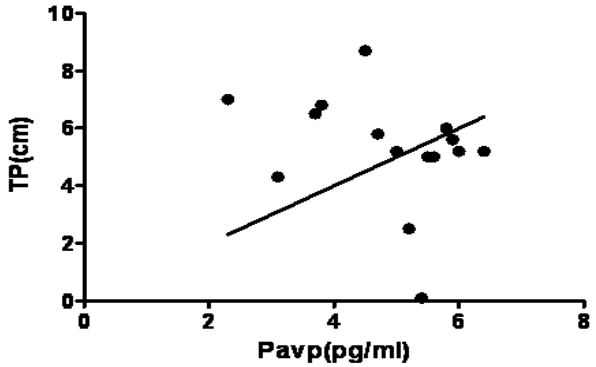

Figure 2.
Relationship between Thirst Perception and Plasma arginine vasopressin in the male group.

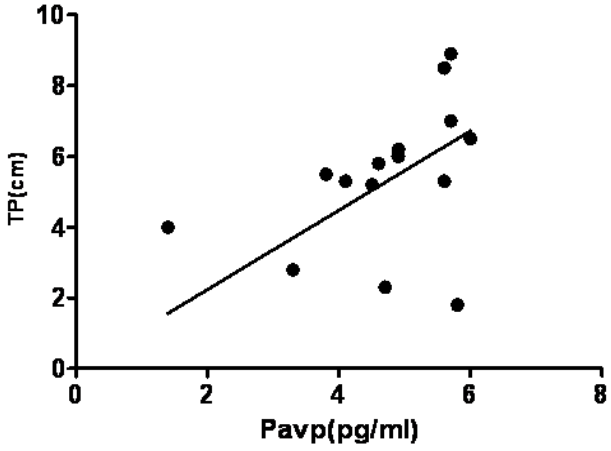

Figure 3.
Relationship between Thirst Perception and Plasma arginine vasopressin in the female group

Table 2:
Thirst perception, Plasma osmolality, measured P_{AVP} and calculated P_{AVP} from TP and P_{osm}, expressed as mean ± SEM, in the male and female subjects.

	TP (cm)	**P_{osm} (mOsm/kg H_2O)**	**P_{AVP} -M (pg/ml)**	**P_{AVP} -TP (pg/ml)**	**P_{AVP} -P_{osm} (pg/ml)**
Male, n = 15	5.26±0.51	298.5±1.71	4.85±0.30	5.40±0.69	6.10±0.70
Female, n = 15	5.39± 0.53	299.0±1.8	4.71±0.31	5.60± 0.70	6.30± 0.80

TP, Thirst perception; P_{osm}, Plasma Osmolality; P_{AVP} –M, measured plasma arginine vasopressin; P_{AVP} –TP, Plasma arginine vasopressin calculated from TP; P_{AVP} -P_{osm} Plasma arginine vasopressin calculated from P_{osm}

Furthermore, the calculated P_{AVP} from TP (Igbokwe and Obika, 2008) were similar (5.40 ± 0.69 vs. 5.60 ± 0.70 pg/ml). When P_{AVP} was calculated from plasma osmolality (Thompson et. al., 1986), the values were also similar (6.10 ± 0.70 vs. 6.30 ± 0.80 pg/ml).

There was a linear relationship between TP (cm) and measured plasma AVP concentrations (pg/ml) in both male and female groups (Figs. 2 and 3), although the correlation was poor. This may be because while the P_{AVP} was measured quantitatively, the sensation of thirst is subjective and not easily quantifiable by scientific measurement (Mckenna and Thompson, 1998).

DISCUSSION

In this study, thirst perception was examined using the visual analogue scale (VAS). This method was developed by Marks et. al., (1988) and has been used with success in the evaluation of several sensory systems (Stachenfeld and Keefe, 2002).

A number of groups have employed this simple technique to measure thirst and have shown in carefully conducted experiments that thirst appreciation does change within the physiological range of plasma osmolalities (Rolls et. al., 1980; Philips et. al., 1984; Thompson et. al., 1986) and the thirst ratings so obtained correlate closely with ambient plasma osmolality. Thirst responses defined by this method are highly reproducible within an individual (Thompson et. al., 1991) and correlate well with the subsequent volume of water drunk (Thompson et. al., 1986).

Tiplady et. al., (1998), established the validity and sensitivity of the Visual Analogue Scale in healthy young and old subjects. The pattern of results obtained did not indicate any marked difference between the age groups in the use of VAS. Both groups proved able to rate both psychological and physical qualities appropriately on such scales without difficulty.

The VAS, though, might be, susceptible to a variety of personal, and cultural influences, this method however provides the best available description of the function of the thirst mechanism so far (Robertson, 1984).

In this study, there was no statistically significant difference, male vs. female in TP (5.26 ± 0.51 vs. 5.39 ± 0.53 cm), calculated plasma osmolality from TP (298.5 ± 1.7 vs. 299.0 ± 1.8mOsm/kgH_2O), and measured plasma arginine vasopressin (4.85 ± 0.30 vs. 4.71 ± 0.31 pg/ml).

The relationship between thirst and plasma osmolality (P_{osm}) has been established by Thompson et. al., (1986), and can be expressed as

$$P_{osm} = \frac{10TP}{3} + 281 \qquad \ldots\ldots\ldots\ldots (1).$$

Although, the above equation was used in calculating the plasma osmolality in this study, the osmolality can be measured using an osmometer or estimated by calculation using the standard formula: Osmolality = 2 x Na + Glucose + Urea (all measurements in mmol/l)

Also the relationship between plasma osmolality and plasma arginine vasopressin (P_{AVP}) was expressed as:

$$P_{osm} = \frac{P_{AVP}}{0.43} + 284.3 \qquad \ldots\ldots\ldots\ldots\ldots..(2)$$

Solving these two equations (1 and 2 above), Igbokwe and Obika, (2008), yielded the equation:

$TP_{cm} = 0.75\ P_{AVP} + 1.2$

The result from their study shows that the above equation relating thirst perception and plasma arginine vasopressin exists and has a mean lower set point of "not thirsty" of 1.2cm mark on the visual analogue scale.

As shown in Figures 1 and 2, a linear relationship exists between TP (cm) and P_{AVP} (pg/ml) in both males and females, although the correlation was poor. This is probably because while the P_{AVP} was measured quantitatively, the TP measurements is subjective and not easily quantifiable by scientific measurement (Mckenna and Thompson, 1998). Although, the thirst perception ratings may be susceptible to a variety of personal and cultural influences (Robertson, 1984), it does change within the physiological range of plasma osmolalities (Rolls et. al., 1980 ; Philips et. al., 1984; Thompson et. al.,

1986) and thirst ratings so obtained correlate closely with plasma osmolality.

Furthermore, plasma arginine vasopressin concentration was calculated from TP using the equation,

$P_{AVP} = \frac{TP - 1.2}{0.75}$ (Igbokwe and Obika, 2008).

The results obtained were similar in both males and females (5.40 ± 0.69 vs. 5.60 ± 0.70 pg/ml).

When plasma arginine vasopressin concentration was calculated from plasma osmolality again as established by Thompson et. al., (1986) i.e., $P_{AVP} = 0.43 (P_{osm} – 284.3)$, the values were also similar (6.10 ± 0.70 vs. 6.30 ± 0.80 pg/ml).

Edwards (1971) reported that the normal plasma AVP concentration measured by immunoassay is about 1 - 5 pg/ml. The Cayman Chemical Company (2011), using the arginine vasopressin Enzyme Immunoassay (EIA) Kit, showed that normal levels of AVP in serum are between 0.4 and 5.2 pg/ml. Studies by Zasshi (1989a and 1989b) showed that enzyme immunoassay for AVP was sufficiently sensitive and specific and the values measured by EIA correlated well with those measured by Radioimmunoassay, and thus EIA could be applied for the determination of physiological levels of AVP in plasma. In this study, Igbokwe and Obika (2008) showed that the threshold for thirst perception using the VAS lies between thirst perceptions of 1.50 - 1.58 cm markings on the VAS, plasma osmolality of 286.0 - 286.3 mOsm/kg H_20 and plasma arginine vasopressin concentration of 0.4 - 0.5 pmol/l (0.45 - 0.55 pg/ml), being the sensitivity of the osmoregulatory unit. Though the values of the calculated plasma AVP concentration in this study were generally higher, the values of the measured plasma AVP in both groups were within the normal ranges, and similar in earlier reports of several other workers (Larose et. al., 1985; Morton et. al., 1975 and Robertson et. al., 1973).

Under physiological conditions, the most important stimulus of vasopressin secretion is an increase in the effective osmotic pressure of the plasma, which may be induced by dehydration, or infusion of hypertonic solutions. Vasopressin secretion is also influenced by haemodynamic factors such as nausea, drugs as well as stress, increased temperature, and angiotensin (Berl and Robertson, 2000).

Increase in plasma osmolality is also the main stimulus for thirst. However, the desire to drink is triggered by an osmotic level that is higher than that leading to the secretion of vasopressin (Robertson, 1984). AVP release begins at an average plasma osmolality of about 280mOsm/kg H_2O, whereas thirst is not perceived until plasma osmolality reaches about 290mOsm/kg H_2O. Thus, during normal living conditions, while vasopressin is constantly present in the blood, the perception of thirst is intermittent. The sensitivity and threshold of the osmoregulatory systems show wide inter-individual variability in both humans and rat (Zerbe et. al., 1991; Bankir, 2001). These individual differences are constant over a prolonged period and appear to be determined mainly by genetic factors (Zerbe et. al., 1991). Since the osmoregulatory mechanisms are not equally sensitive in all individuals, one can expect some subjects to tend to be continuously in a state of slight dehydration, and thus to have a high level of vasopressin in circulation.

Studies in sheep have suggested that the cerebral osmoreceptors subserving thirst and vasopressin secretion were, at least in part, located in brain regions lacking a blood-brain barrier (Mckinley et. al., 1978). Subsequently, evidence from the study of cerebral lesions, electrophysiological recordings, and the expression of the immediate early gene, c-*fos*, in rats have confirmed that neurons in both the Organum Vasculosum of the Lamina Terminalis (OVLT) and the Subfornical organ (SFO), which are located in the anterior wall of the third ventricule are mostly likely the sites of very sensitive osmoreceptors (Mckinley, et. al., 2003). In a normal situation, AVP acts along with thirst to maintain serum osmolality within a narrow range (Dhanidharka and Langman, 2009). Anything that stimulates AVP secretion also stimulates thirst, e.g., the non-osmotic switch-off of thirst during the act of drinking is also very similar to that of vasopressin, with a fall in thirst ratings before significant changes in plasma osmolality occur (Thompson et. al., 1987; Seckle et. al., 1986).

In summary, there was no statistically significant difference between the P_{AVP} measured as well as calculated from TP and from plasma osmolality. This work therefore is found to validate the findings and equations put forward by Igbokwe and Obika, (2008) and Thompson et. al., (1986), and further establishes that there is a linear relationship between plasma arginine vasopressin and thirst.

It is reasonable to conclude that plasma arginine vasopressin concentration may be estimated using thirst perception and/or plasma osmolality values.

Part of this work has been presented to the Physiological Society of London (C07 and PC07) at a themed meeting held at the Royal Free Campus of the University College, London, on 1 – 3rd September, 2011.

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