Diurnal Variation of Plasma Arginine-Vasopressin in Pregnant and Non-Pregnant Goats *(Capra hircus)*

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Received April 7, 2004
Accepted March 3, 2005

Abstract

Skotnicka E.: Diurnal Variation of Plasma Arginine-vasopressin in Pregnant and Non-pregnant Goats *(Capra hircus)*. Acta Vet. Brno 2005, 74: 43-49.

The aim of this study was to estimate and analyse daily profiles of plasma arginine-vasopressin concentration (AVP), sodium, potassium, chloride concentrations and the plasma osmolality (PO) in pregnant (n = 9) and non-pregnant (n = 17) goats. The influence of late pregnancy on AVP diurnal changes and correlation between PO and electrolyte concentrations were determined.

Plasma AVP in both groups of goats changed during 24 h, with the highest values in the dark phase and lowest values during the light phase. AVP in pregnant goats ranged from 0.66 $fmol \cdot ml^{-1}$ to 1.14 $fmol \cdot ml^{-1}$ and was higher than that in non-pregnant goats (0.48–0.83 $fmol \cdot ml^{-1}$), especially during the dark phase. Relatively stabilised concentration of osmotic active electrolytes in plasma in both groups of goats suggests the efficiency of the processes protecting the water-electrolyte homeostasis, which is especially significant in pregnancy.

Diurnal variations, arginine-vasopressin, plasma osmolality, electrolytes, pregnancy

Diurnal changes have been observed in many physiological variables. Renal excretion of salt and water in various species of mammals is known to show clear patterns over the 24 h cycle (Muszczyński 1996; Voogel et al. 2001). One might also expect to find parallel variations in the secretory or plasma concentration pattern of the hormones controlling water-electrolyte metabolism. Among these hormones, arginine-vasopressin (AVP) plays a well-established role in controlling salt and water excretion, volume of water spaces and blood pressure. The role of AVP in biological clock system (Mihai et al. 1994) and its influence on secretion and synthesis of melatonin in the pineal gland (Simonneaux et al. 1996) is still discussed.

Water-electrolyte metabolism undergoes significant modifications in pregnancy (Koehler 1993; Lindheimer and Davison 1995). It is connected with altered activity of regulating systems and with changes in the intensity of metabolic processes. Hence it may be assumed that the antidiuretic system activity also undergoes certain changes in pregnancy.

Diurnal changes in plasma vasopressin concentration and plasma osmolality are not well known in ruminants, especially in goats. In humans, diurnal variation with higher plasma AVP concentration at night has been reported by Asplund and Aberg (1991), Aikawa et al. (1999), Forsling (2000). In rats, which are nocturnally active animals, plasma AVP has been reported to vary with peak in the evening, at the beginning of the dark period (Terwel et al. 1992; Windle et al. 1992).

The aim of the present study was to measure and analyse diurnal variations in plasma AVP concentration, plasma osmolality (PO) and electrolytes concentrations in goats. Moreover, the influence of late pregnancy on the diurnal changes of antidiuretic system was examined.

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Materials and Methods

Animals

The study was carried out in January and February. The studies were performed on 26 clinically healthy goats (75% of the Polish White Shorthaired Breed) aged 2-3 years. The animals were divided into 2 groups, depending on their physiological state. First group (n = 17) represents non-pregnant, non-lactating goats, second group (n = 9) and goats in late pregnancy, 2 weeks before first parturition. The animals of both groups were kept under controlled temperature (about 15 °C) and lighting with simulated-natural photoperiod (9h light/15h darkness, light off: 16:30-07:30 h.

For 10 d prior to the studies and also during the studies the animals the particular study groups were kept in two separate parts of the room. They were fed according to the standards (barley grain 600 g per day, beetroot pulp 400 g per day; water, hay and salt-lick to taste). The dry food was given at 09:00 h. Before the experiment began, the external jugular vein of animals was catheterized to enable blood sampling without stress.

Analytical procedure

Blood was collected from the external jugular vein, at the time of sampling at 16:00, 20:00, 24:00, 04:00, 08:00, 12:00, 16:00 h.

After 24 h the scheme was repeated. While blood was collected in the dark phase, a red spotlight was used. Blood samples (5 ml) were collected into tubes containing EDTA- thylenedinitrilotetraacetic acid disodium salt dihydrate (2 mg·ml^{-1} of blood) for plasma AVP analysis or heparine (250 I.U. Heparine, Heparinum Jelfa Poland) for plasma electrolyte and PO analysis. The samples were centrifuged at 2000 × g for 30 min. Plasma samples were stored at –20 °C prior to analysis of plasma AVP and electrolytes concentrations, or kept on ice prior PO determination.

Biochemical Procedure

Plasma arginin-vasopressin concentration (AVP) was measured by RIA Kit, code 23065, INCSTAR Co. – Minnesota, USA (based on the measurement of vasopressin with iodine [Vasopressin125I]), with intra- and interassay coefficients of variation of 8.2% and 11.8%, respectively. The sensitivity of the vasopressin assay was 0.24 fmol·ml^{-1}.

Plasma osmolality (PO) was determined immediately after experiments by cryoscopic method using a Knauer osmometer. Plasma sodium and potassium concentrations were measured by flame photometry using a Flapho-4 photometer. Plasma chloride concentration was determined by potentiometric titration using a Spexon –100 Chlorimetr.

Statistical analysis

Values are presented as means ± SD. For multiple comparisons, the analysis of variance (ANOVA) followed by Student's *t*-test was used. Significant differences between means for paired samples studies were identified using Student's paired *t*-test. Significance was taken as $P < 0.05$.

Results

Plasma AVP concentration in the non-pregnant goats ranged between 0.48 fmol·ml^{-1} to 0.83 fmol·ml^{-1} (Fig. 1). The highest AVP concentrations were observed in the evening and at night. The differences between the maximum values at 16:00, 20:00 and 24:00 and the minimum at 8:00 were statistically significant ($P < 0.05$). The AVP concentration in the pregnant goats also varied during the 24 h period. The maximum concentration of AVP (1.14 fmol·ml^{-1}) was observed at 24:00. In the morning AVP level decreased, reaching the minimum values (0.66 fmol·ml^{-1}) at 12:00. During the night and in the morning, the AVP levels in pregnant goats were significantly higher, than those in non-pregnant animals (24:00 and 4:00, $P < 0.05$). Plasma osmolality (PO) in the non-pregnant and pregnant goats is presented in Fig. 2. In non-pregnant animals, the lowest PO value was observed at 24:00, 4:00 and 8:00 and the highest at 16:00. In pregnant goats the differences between minimum (at 4.00 and 8.00) and maximum values (16:002 and 20:00) were statistically significant ($P < 0.05$ and $P < 0.02$, respectively). In non-pregnant goats, correlation between plasma AVP concentration and PO for five collecting times was significant (16:00: r = 0.68, $P < 0.001$; 20:00: r < 0.05, $P < 0.001$; 24:00: r = 0.52, $P < 0.05$; 4.00: r =0.60, $P < 0.01$; 12.00: r = 0.61, $P < 0.05$). In the pregnant goats only at 4.00 the correlation was significant (r = 0.68, $P < 0.05$).

Plasma Na^+ concentration in both groups of goats was relatively stable (Table 1), but in the pregnant goats increase of Na^+ concentration was observed at the end of experiment. Na^+ concentrations in the pregnant goats were significantly lower than those in the non-pregnant animals. Plasma K^+ and Cl^- concentrations in both groups of goats did not change during

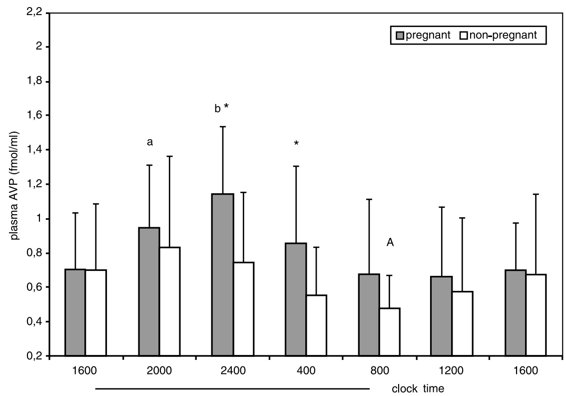

Fig. 1. Diurnal variations in plasma AVP concentration in non-pregnant (n = 17) and pregnant (n = 9) goats. Values are hourly means ± SD.* significance of differences between the mean values of plasma AVP concentration in both groups at the particular time ($P < 0.002$ vs 24:00, $P < 0.0005$ vs 04:00). Significance of differences between the mean values of plasma AVP concentration in non-pregnant goats: (A) $P < 0.05$ vs 16:00 h, 20:00 h, 24:00 h and pregnant goats: (a) $P < 0.05$ vs 16:00 h, (b) $P < 0.05$ vs 4:00 h, 8:00 h, 12.00 h, 16.00 h. Underlined represents the night period.

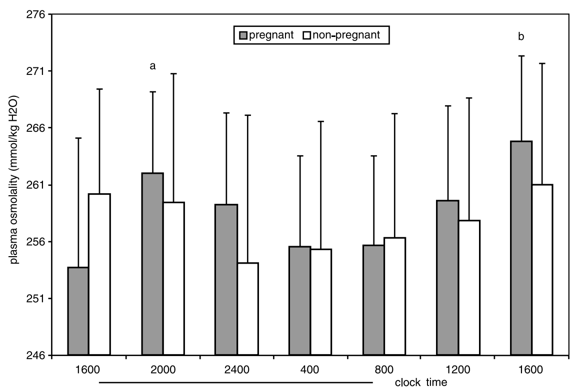

Fig. 2. Diurnal variations in plasma osmolality in non-pregnant (n = 17) and pregnant (n = 9) goats. Values are hourly means ± SD. *significance of differences between the mean values of plasma osmolality in pregnant goats: (a) $P < 0.05$ vs 4:00, 8:00, (b) $P < 0.02$ vs 4:00, 8:00. Underlined represents the night period.

experiment (Table 1). At the beginning of the light phase, K^+ and Cl^- values in pregnant goats were significantly lower, than in non-pregnant ($P < 0.05$ and $P < 0.005$, respectively). In non-pregnant goats positive correlation between plasma AVP and K^+ concentration occurred at 20:00 (r = 0.60, $P < 0.01$) and 24:00 (r = 0.71, $P < 0.01$).

Discussion

In the present study, the mean of AVP plasma level in both experimental groups was similar to that reported in goats by Kokkonen et al. (2001). Hormone concentrations were elevated over the dark hours of the cycle, when the goats were relatively inactive. It was also found in calves (Wolf et al. 1991) rats (Terwel et al. 1992; Windle et al. 1992) and in human (Aikawa et al. 1999; Asplund and Aberg 1991; Forsling 2000). However, Reppert et al. (1981) in cats, Perlov et al. (1982) in monkeys and Kokkonen et al. (2001) in anoestrous goats did not find any circadian variations in AVP concentrations in plasma.

Table 1.Diurnal variations in concentrations of sodium (a), potassium (b) and chlorides (c) in plasma of the non-pregnant (n = 17) and pregnant goats (n = 9). Results as mean values ± SD.

Time	Na^+ ($mmol \cdot l^{-1}$)			K^+ ($mmol \cdot l^{-1}$)			Cl^- ($mmol \cdot l^{-1}$)		
	Non-pregnant	pregnant	P-value*	non-pregnant	pregnant	P-value*	non-pregnant	pregnant	P-value*
16.00_1	140 ± 7	130 ± 8	< 0.002	3.6 ± 0.2	3.5 ± 0.5	< NS	107 ± 5	105 ± 6	< NS
20.00	**140 ± 6**	**131 ± 7**	**< 0.0002**	**3.7 ± 0.4**	**3.7 ± 0.3**	**< NS**	**107 ± 6**	**107 ± 6**	**<NS**
24.00	**138 ± 5**	**130 ± 5 ##**	**< 0.00003**	**3.6 ± 0.6**	**3.8 ± 0.3**	**< NS**	**106 ± 6**	**109 ± 5**	**<NS**
4.00	**138 ± 3**	**132 ± 7**	**< 0.004**	**3.7 ± 0.4**	**.3.7 ± 0.2**	**< NS**	**109 ± 7**	**107 ± 6**	**<NS**
8.00	138 ± 5	131 ± 6	< 0.004	3.8 ± 0.5	3.5 ± 0.5	< 0.05	108 ± 5	104 ± 5	<0.005
12.00	136 ± 5 #	136 ± 8	< NS	3.8 ± 0.4	3.5 ± 0.5	< NS	106 ± 5	105 ± 7	<NS
16.00_2	137 ± 4	137 ± 7	< NS	3.6 ± 0.6	3.6 ± 0.3	< NS	106 ± 4	108 ±	<NS

**P*-values for comparison between non-pregnant and pregnant
# $P < 0.03$ vs $16{:}00_1$, 20:00
$P < 0.05$ vs $16{:}00_2$.
Bold text represents the night period.

The different results obtained in these species might be related to their different periods of activity, postural changes, and/or physiological state. In diurnally active subjects, sleeping in supine position, plasma AVP has been reported to increase at night (Aikawa et al. 1999; Asplund and Aberg 1991; Forsling 2000). In night active rats, AVP concentrations were elevated during the day (Terwel et al. 1992; Windle et al. 1992).

In contrast to our study Kokkonen et al. (2001) demonstrated the absence of circadian variation in plasma AVP concentration in anoestrous goats. In the present study, we did not record behaviour continuously and therefore we cannot exactly define the number of rest-activity periods during the experiment. In our experience, however, the goats more often rested at night than in the daytime. It is noteworthy that our goats were able to lie down, stand up, and eat and drink freely during experiment. In a study carried out by Kokkonen et al. (2001), goats during sampling were restricted in metabolism cages.

The main factors influencing the synthesis of AVP in the hypothalamus and its secretion from the neural part of pituitary gland are osmotic and volumetric stimuli. Windle et al. (1992) suggest that the changes in plasma AVP concentration during 24 h period are similar to the changes of diuresis and excretion of electrolytes. However, in our study we did not measure haemodynamics and renal function, but in non-pregnant animals a correlation between AVP concentration and the plasma osmolality has been shown. Thus it seems that in non-pregnant goats, plasma osmolality is the main factor, which affects plasma AVP concentration.

In the present study PO in non-pregnant group changed during the 24 h period. Skotnicka et al. (1997) in calves, did not observed diurnally changes of plasma osmolality. However, Kanervo et al. (1991) and Windle et al. (1992) observed circadian changes of plasma osmolality in rats, with the highest values around midnight, in the middle of animals' activity period. Asplung and Aberg (1991) in human and Windle et al. (1992) in adult rats did not find correlation between circadian variations in plasma osmolality and AVP.

Despite the fact that the tendency of variations of AVP concentration in pregnant goats was almost the same as in non-pregnant goats, the maximum was 3 h later. In the present study plasma AVP concentration in pregnant goats was significantly higher at 24:00 and 4:00 than that in non-pregnant goats. The increase of AVP concentration in late pregnancy was also observed by other authors in sheep, cows, hamsters, mice, rats and women (Keller–Wood 1994; Keller–Wood 1995; Koehler 1993; Lindheimer and Davison 1995). It seems that in pregnancy the higher AVP concentration contributes to an increase in plasma volume and stabilization of plasma osmolality. Keller-Wood (1994, 1995) and Koehler (1993) suggest that the pregnancy does not modify significantly the mechanism of AVP secretion induced by changes in Na concentration and/or other osmotic active substances. There also may be other factors responsible for higher AVP in pregnancy, as an increase in plasma renin activity and the consequent increase in concentration of angiotensin II and/or prostaglandines, which intensify the synthesis of AVP (Skotnicka 2003). It could be also due to the lower sensitivity of baroreceptors and decreased arterial blood pressure (Baylis 1994). Keller-Wood (1994) has observed a lack of any clear relationship between the concentration of AVP in plasma and the variations in arterial blood pressure of pregnant sheep, on the contrary to non-pregnant ones where the relationship has been close. Many authors suggest that during pregnancy plasma osmolality decreases (women, rats) by about 10 mosmol·kg^{-1}, remaining on this level until delivery (Baylis 1994; Keller–Wood 1994; Koehler 1993; Lindheimer and Davison 1995). In contrast, in our study plasma osmolality in pregnant goats in the afternoon and during the night was higher than that of the non-pregnant goats; the differences, however, were not statistically significant.

In the present study, plasma Na^+ concentration in the non-pregnant and pregnant goats was relatively stable during the 24 h period similar to results by Muszczyński et al. (1996) in pregnant goats. Yet Kokkonen et al. (2001) in anoestrous goats, Skotnicka et al. (1997) in calves and Kanabrocki et al. (1973) and Sothern et al. (1996) in man observed circadian variations in plasma sodium concentration. In the present study, no diurnal variation in plasma K^+ concentration in both groups of goats was found, similar to that by Kokkonen et al. (2001) in anoestrous goats. However, diurnal variations in the plasma K^+ concentration were observed in calves (Skotnicka et al. 1997), in pregnant goats (Muszczyński et al. 1996) and in humans (Kanabrocki et al. 1973). Moreover, Solomon et al. (1991) observed an increase in the plasma K^+ concentration during the day, with the maximum around noon. In the present study, plasma Cl^- concentration in non-pregnant and pregnant goats remained relatively stable. Similarly Muszczyński et al. (1996) in pregnant goats and Skotnicka et al. (1997) in calves did not find any diurnal variations in Cl^- plasma concentrations.

In conclusion, the results of the present study show that in both pregnant and non-pregnant goats, plasma AVP concentrations changed diurnally with achieved highest values at night, and overall higher activity of the AVP system in goats pregnant at this time. Relatively stabilised concentration of osmotic active electrolytes in plasma in both groups of goats suggests the efficiency of the processes protecting the water-electrolyte homeostasis, which is especially significant in pregnancy.

Změny plazmatické koncentrace argininu-vazopresinu v průběhu dne u březích a nebřezích koz (*Capra hircus*)

Cílem studie bylo stanovit a analyzovat denní profil plazmatické koncentrace argininu-vazopresinu (AVP), Na, K,Cl a osmolaritu plazmy (PO) u březích (n = 9) a nebřezích (n = 17) koz. Byl určen vliv pozdní gravidity na změny AVP v průběhu dne a vztah mezi PO a koncentrací elektrolytů.

Plazmatická AVP se u obou skupin koz během 24 h měnila, hodnoty byly nejvyšší

v tmavé a nejnižší ve světlé fázi dne. AVP se u březích koz pohybovala od 0,66 fmol·ml^{-1} do 1,14 fmol·ml^{-1} a byla vyšší než u zvířat nebřezích (0.48–0.83 fmol·ml^{-1}) a to zejména v tmavé fázi dne. Poměrně stálé koncentrace osmoticky aktivních elektrolytů v plazmě u obou skupin koz svědčí o efektivnosti procesů udržování eletrolytové homeostáze, která je v graviditě zvlášť významná.

Acknowledgment

Author wishes to thank Professor W. F. Skrzypczak, Dr. Z. Muszczyński and people from the Department of Animal Physiology Agricultural University of Szczecin for all facilities and friendly atmosphere during the experiments. Special thanks go to Professor E. Kulczykowska (Institute of Oceanology of Polish Academy of Sciences, Gdynia) and Professor K. Janus (Agricultural University of Szczecin) for critically reading the manuscript and providing helpful advice.

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