Atrial Natriuretic Peptide and Renal Haemodynamics in Newborn Calves

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Abstract

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The study was aimed to provide information on the dynamics of changes in the concentration of ANP in blood plasma of calves during the first seven days of their life and to find any association between blood plasma ANP concentration and effective renal blood (ERBF) and plasma flow (ERPF) and glomerular filtration rate (GFR). The experiment was carried out on 10 clinically healthy Black and White calves during the first seven days of postnatal life. The concentration of atrial natriuretic peptide (ANP) in blood plasma was determined with radioimmunoassay. The kidney function was assessed by clearance methods, using para-aminohippuric acid and inulin. The blood plasma ANP concentration increased with age. On the first day the mean concentration of ANP was 5.72 ± 1.34 pmol/l, on the 7th day it was 14.34 ± 2.09 pmol/l. The ERBF and ERPF during the first week of life showed variations, mean ERBF ranging within 280.22 ± 10.30 ml/min/m^2 (1st day) and 338.25 ± 13.72 ml/min/m^2 (7th day) and ERPF ranging between 179.77 ± 8.27 ml/min/m^2 (1st day) and 245.22 ± 9.86 ml/min/m^2 (7th day). The mean GFR on the first day was 35.68 ± 2.10 ml/min/m^2 and significantly ($p \leq 0.01$) increased on the second day 49.97 ± 1.76 ml/min/m^2, subsequently GFR remained on a stabile level average 51.06 ml/min/m^2. The studies showed no correlations between plasma ANP concentration and ERPF (ERBF) and GFR. These data indicate that in calves during the first seven days of postnatal life endogenous ANP did not change the effective renal blood (plasma) flow and glomerular filtration rate.

ANP, kidneys, renal blood (plasma) flow, glomerular filtration rate

Atrial natriuretic peptide (ANP) is synthesised, stored, and released primarily by the myocytes of the atrium cordis. Increased blood volume and/or blood hydrostatic pressure contribute to the release of ANP to the circulation (Chapman et al. 1998; Chevalier et al. 1990a; Grant et al. 1996; Takemura et al. 1990).

It has been demonstrated that atrial natriuretic peptide receptors are located in many organs, including blood vessels, adrenal glands, pituitary gland, cerebellum, heart, small and large intestines; however, their highest number has been found in kidneys, i.e. in renal glomeruli, mesangium, the vessels of microcirculation, as well as in renal tubules (Chai et al. 1986; Healy and Fanestil 1986; Lynch et al. 1986; Rutherford et al. 1994; Takeda et al. 1986; Wilcox et al. 1991).

Atrial natriuretic peptide may influence kidneys directly, affecting the functioning of the nephrons, or indirectly, modifying the haemodynamics of the kidney. It has been recognised that ANP, through influence on glomerular afferent and efferent arterioles, may also affect renal blood (and plasma) flow and glomerular filtration rate (Dunn et al. 1986; Marin-Grez et al. 1986). However, the literature provides discrepant information on the influence of ANP on renal haemodynamics (Abu-Amarah and Balment 1999; Eiskjaer et al. 1996; Garcia-Estan and Roman 1990; Janssen et al. 1994; Semmekrot et al. 1990). It has also been published that an effect of exogenous ANP on renal blood (and plasma) flow and glomerular filtration rate may depend on the age of the examined individuals (Chevalier et al. 1988; Robillard et al. 1988; Semmekrot et al. 1990).

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This study was aimed to provide information on the dynamics of changes in the concentration of atrial natriuretic peptide in blood plasma of calves during the first seven days of their life and to find any association between blood plasma ANP concentration and effective renal blood and plasma flow and glomerular filtration rate.

Materials and Methods

The experiment was carried out on 10 clinically healthy Black-and-White calves during the first seven days of postnatal life. After the necessary zootechnical procedures and feeding colostrum, the calves were transported to the research facility of the Department of Animal Physiology. During the experiment, the animals remained in individual boxes in equal environments. The calves were fed colostrum and 6 - 7 litres of maternal milk per day (three times a day).

Before the experiment, Foley balloon catheters had been inserted into the urinary bladders of the calves (each day of the experiment) and their external jugular veins were catheterised. The calves were weighed each day before the samplings that always took place at the same time of the day, one hour after morning feeding (at 9.00 h).

The kidney function was analysed by means of clearance tests, using administered test substances. Para-aminohippuric acid (Sigma Chemical Company, St. Louis, MO, USA) was used to measure renal plasma flow, while inulin (Sigma Chemical Company, St. Louis, MO, USA) was used to diagnose plasma glomerular filtration rate.

The analyses started with the collection of a blood (K_0) and urine (U_0) sample, which were used as "blind" tests, necessary for further analyses. Thereafter, the calves were intravenously administered 2.5 g inulin (in 20 ml of 0.9% NaCl), and 20 minutes after inulin administration, 1.5 g of para-aminohippuric (in 10 ml H_2O) was injected. Forty minutes after the inulin injection, urine was collected to polyethylene bags within two periods of time in order to measure per-minute urine flow rate. In the middle of each urine sampling period, blood was collected to ETDA tubes (Vacuette with 1.8 mg ETDA-K_3 per 1 ml blood; Greiner Bio- One, Austria), to measure ANP concentration, and to heparin tubes (Heparin, Biochemie, Austria) in order to assay the test substances. Immediately after the blood samplings, haematocrit was measured, which was used in further analyses to calculate renal blood flow. The collected blood samples were immediately centrifuged at 3000 rpm at 4 °C for 15 min. The resulting plasma was stored at -20 °C until further analyses.

Plasma was assayed for ANP and test substances concentrations, while the concentrations of inulin and para-aminohippuric acid were measured in urine. The assays were performed with the following methods:

Atrial natriuretic peptide - radioimmunoassay, based on concentration measurements of ^{125}I-labelled ANP, using a Shionoria ANP kit, CIS Bio Intl, France (Clerico 1996).

Haematocrit - micromethod.

Inulin - resorcinol method (Borkowski et al. 1986).

Para-aminohippuric acid - modified Brun's method (Waugh and Beall 1974).

In order to comprehensively diagnose the functioning of kidneys, the following were calculated:

effective renal blood flow (ERBF),

clearance of inulin (C_{in}) and para-aminohippuric acid (C_{PAH}).

$$C = \frac{U \cdot V}{P} \text{ [ml/min]}$$

$$ERBF = \frac{ERPF}{1\text{-}Ht} \text{ [ml/min]}$$

where: C - clearance,
U - urine concentration [mmol/l],
P - blood plasma concentration [mmol/l],
V - diuresis [ml/min],
ERBF - effective renal blood flow,
Ht - haematocrit [1/1],
ERPF - effective renal plasma flow (C_{PAH}) [ml/min],
GFR - glomerular filtration rate (C_{in}) [ml/min],

The obtained data were standardised per 1 m^2 of calf body surface area, according to Meeh's formula (after Ketz 1974):

$$S = 0.105 \cdot \sqrt[3]{bw^2} \ [m^2]$$

where: *S* - body surface area;
bw - body weight.

From the raw results obtained, means and standard deviations were calculated. In order to estimate significance of differences, ANOVA with replications was employed. Relationships between the concentration of atrial natriuretic peptide in blood plasma and tested indices of renal function in the subsequent days of calves' postnatal life were estimated using coefficients of correlations. Statistical computations were performed by means of Statistica 6.0 software package.

Results

Over the first seven days of life, blood plasma ANP concentration in the calves was found to increase significantly ($p \leq 0.01$). On the first day postpartum, the mean concentration of this hormone was 5.72 ± 1.34 pmol/l, while on the 7th day it was 14.34 ± 2.09 pmol/l. The highest blood plasma ANP concentration was recorded on the sixth day (mean 16.43 ± 1.92 pmol/l). The mean ANP concentration in blood plasma for the first week of life was 11.40 pmol/l (Table 1).

Table 1. Concentration of atrial natriuretic peptide (ANP), effective renal blood flow (ERBF), effective renal plasma flow (ERPF), and glomerular filtration rate (GFR), diuresis (V)

Item		Day of life							Significance of differences	
		1	*2*	*3*	*4*	*5*	*6*	*7*	$p \leq 0.01$	$p \leq 0.05$
ANP	$\bar{x}$ [pmol/l]	5.72	9.39	10.28	11.27	12.35	16.43	14.34	*1 → 4-7* *2 → 6* *3 → 6* *4 →*	*1 → 3* *2 → 7* *5 → 6*
	SD	4.23	6.60	5.49	4.82	4.47	6.09	6.60		
ERBF	$\bar{x}$ [ml/min/m^2]	280.22	310.06	336.11	306.09	289.56	313.62	338.25	*1 → 3, 7* *3 → 5* *5 → 7*	*1 → 2, 4, 6* *2 → 5* *3 → 4* *4 → 7* *5 → 6*
	SD	32.57	26.00	33.15	18.11	18.81	29.49	43.40		
ERPF	$\bar{x}$ [ml/min/m^2]	179.77	211.17	234.82	217.71	209.65	227.22	245.22	*1 → 2–7* *3 → 5* *5 → 7*	*2 → 3, 7* *3 → 4* *4 → 7* *5 → 6*
	SD	26.14	21.92	21.81	18.89	17.65	22.15	31.17		
GFR	$\bar{x}$ [ml/min/m^2]	35.68	49.97	52.43	49.33	53.01	49.98	51.67	*1 → 2–7*	*3 → 4*
	SD	6.63	5.56	4.51	7.41	7.33	6.20	5.89		
V	$\bar{x}$ [ml/min/m^2]	0.51	0.80	1.41	1.17	1.63	1.36	1.42	*1 → 3–6* *2 → 5*	*2 → 3 ,4, 6* *4 → 5*
	SD	0.28	0.43	0.82	0.57	0.58	0.38	1.16		

The mean renal blood flow (ERBF) and mean plasma flow (ERPF) during the first week of life remained at a low level and showed variations, mean ERBF ranging within 280.22 ± 10.30 and 338.25 ± 13.72 ml/min/m^2 and ERPF ranging between 179.77 ± 8.27 and 245.22 ± 9.86 ml/min/m^2 (Table 1). The values of these indices differed significantly ($p \leq 0.05$) between individual days of the experiment. What should be emphasised, both renal blood and renal plasma flow increased significantly ($p \leq 0.01$) within the first three days. Changes in ERBF between individual days were correlated ($p \leq 0.01$) with those in ERPF. No statistically significant correlations ($p \leq 0.05$) were found between changes in ANP concentration and ERBF or ERPF.

The observed average glomerular filtration rate during the first seven days of life in calves was 48.87 ml/min/m^2 (Table 1). The mean GFR was the lowest during the first day, 35.68 ± 2.10 ml/min/m^2. The mean glomerular filtration rate was observed to significantly ($p \leq 0.01$) increase on the second day, up to 49.97 ± 1.76 ml/min/m^2, to stabilise over the remaining days of the first week of life at a mean level of 51.06 ml/min/m^2. It should be noted that no correlation ($p \leq 0.05$) was found during the first week of life between the renal blood (plasma) flow and the glomerular filtration rate.

Discussion

The increase in the concentration of ANP in blood plasma with age has been confirmed also by Tulassay et al. (1987), who reported a low concentration of atrial natriuretic peptide in the first hour postpartum (22.1 fmol/l) in human neonates, followed by a significant increase in ANP concentration until the third day of life (45.2 fmol/l). Also, Gemelli et al. (1991) found a significant increase in ANP concentration in the blood plasma of human neonates within the first 24 hours of life.

A reverse trend in the changes in atrial natriuretic peptide concentration in blood plasma of calves was observed by Takemura et al. (1994). The highest concentration of the hormone was recorded after birth (62.7 pmol/l), while the lowest on the 10^{th} day of age (11.3 pmol/l). Also Amadieu-Farmakis et al. (1988a; 1988b) observed the highest ANP concentration in calf blood plasma directly upon birth (20 pmol/l on average) and a decrease in the concentration until the third day of age (16.5 pmol/l). In the remaining days of the first week of life, the authors found a slightly growing trend.

A number of factors may have influenced the increase in blood plasma ANP concentration with age of the calves observed in our experiment. It is known that the main factors causing an increased release of atrial natriuretic peptide include increased volume and/or pressure of circulating blood (Chapman et al. 1998; Chevalier et al. 1990a; Grant et al. 1996; Takemura et al. 1990). Chevalier et al. (1990b) have demonstrated that the renal response to ANP during the neonatal period in rats, after an experimental increase in blood plasma volume, increases with age. It has also been demonstrated that the total water volume in a calf during the first week of life is high and reaches on average 80% of the body weight (Skrzypczak 1991).

The increase in atrial natriuretic peptide concentration in blood plasma during the first week of neonatal life of calves observed in our experiment may have probably resulted from enhanced synthesis and release of ANP. This is confirmed by the results reported by Silberbach et al. (1991), who studied one- and seven-day-old lambs.

Available literature does not provide clear information on a possible effect of atrial natriuretic peptide on renal blood or plasma flow. Neither does it inform on a possible reverse effect of changes in ERBF or ERPF on ANP concentration in blood. It is possible that the reasons for the increased renal blood and plasma flow observed in our experiment during the first three days of life may include elevated ANP concentration in blood plasma; however the coefficient of correlation between these indices proved non-significant ($p \leq 0.05$). This suggestion has been confirmed by Nushiro et al. (1987), Seino et al. (1988), who studied adult rabbits, and Kimura et al. (1986), who experimented on adult dogs, and who have demonstrated that intravenously administered exogenous ANP triggers a growth in ERBF (ERPF). Using a similar experimental design, Eiskjaer et al. (1996) and Janssen et al. (1994) in adult humans, as well as Semmekrot et al. (1990) in rabbits, observed these indices to decrease. On the other hand, no changes in renal blood (plasma) flow were found by Brown and Corr (1987) in humans, and by Dunn et al. (1986) and Garcia-Estan and Roman (1990) in adult rats.

So far, it has not been clearly established whether and how atrial natriuretic peptide affects renal plasma filtration (Abu-Amarah and Balment 1999; Bidiville et al. 1988; Chevalier et al. 1988; Garcia-Estan and Roman 1990; Janssen et al. 1994; Robillard et al. 1988; Seino et al. 1988; Semmekrot et al. 1990). In our experiment, a lack of relationship was demonstrated between changes in ANP concentration in blood and glomerular filtration rate (coefficient of correlation non-significant at $p \leq 0.05$). This also shows that a statistically significant increase in ANP concentration in the blood plasma of calves on the sixth day of life did not cause a significant change in the glomerular filtration rate. Lack of effect of ANP on GFR has been also confirmed in studies on adult humans (Bidville et al. 1988), dogs (Kimura et al. 1986), rats (Abu-Amarah and Balment 1999, Garcia-Estan and Roman 1990), lambs (Robillard et al. 1988), and young rats (Chevalier et al. 1988). The above-mentioned results may support the suggestion by

Granger (1998) that the lack of changes in the glomerular filtration rate results from ANP "redirecting" blood into the medullary part of the kidneys. Conversely, Janssen et al. (1994) in adult humans, Robillard et al. (1988) in adult sheep, Seino et al. (1988) in adult rabbits, and Chevalier et al. (1988) in adult rats, observed an increased glomerular filtration rate after administration of exogenous ANP. According to Dunn et al. (1986) and Marin-Grez et al. (1986), enhanced GFR may be a result of haemodynamics changes in the blood vessels of the glomeruli which lead to elevated glomerular filtration pressure. Conversely, Semmekrot et al. (1990) observed decreasing GFR in young (4 - 11 days old) and adult rabbits.

The results of this study show that in calves during the first seven days of postnatal life endogenous ANP did not change the effective renal blood (plasma) flow and glomerular filtration rate.

Natriuretický peptid a hemodynamika v ledvinách u novorozených jehňat

Cílem studie bylo prozkoumat dynamické změny v koncentraci atriového natriuretického peptidu (ANP) v krevní plazmě u jehňat během prvních sedmi dní po narození a prostudovat vztah mezi koncentrací ANP v krevní plazmě, průtokem krve (ERBF) a krevní plasmy (ERPF) ledvinami, rychlostí glomerulární filtrace (GRF). Pokus byl proveden na deseti klinicky zdravých, černobílých jehňatech během prvních sedmi dní po narození. Koncentrace ANP v krevní plazmě byla zjišťována pomocí radioimunoanalýzy (RIA). Funkce ledvin byla hodnocena pomocí clearance s využitím kyseliny paraaminohippurové a inulinu. Koncentrace ANP v plasmě se s věkem zvyšovala. První den byla průměrná koncentrace ANP 5,72 ± 1,34 pmol·l^{-1}, sedmý den pak 14, 34 ± 2,09 pmol·l^{-1}. ERBF a ERPF se během prvního týdne života měnily. Průměrný ERBF se pohyboval v mezích 280,22 ± 10,30 ml·min^{-1}·m^{-2} (1. den) a 338,25 ± 13,72 ml·min^{-1}·m^{-2} (7. den), ERPF pak v mezích 179,77 ± 8,27 ml·min^{-1}·m^{-2} (1. den) a 245,22 ± 9,86 ml·min^{-1}·m^{-2} (7. den). Průměrná GRF byla první den 35,68 ± 2,10 ml·min^{-1}·m^{-2} a signifikantně se zvýšila druhý den: 49,97 ± 1,76 ml·min^{-1}·m^{-2} (p L 0,01), následující dny se stabilizovala na průměrné hodnotě 51,06 ml·min^{-1}·m^{-2}. Studie ukázaly, že neexistuje žádný vztah mezi koncentrací ANP v plazmě, ERPF (ERBF) a GRF. Tyto výsledky nasvědčují tomu, že během prvního týdne po narození endogenní ANP u jehňat neovlivňuje průtok krve (krevní plazmy) ledvinami ani rychlost glomerulární filtrace.

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