ACTA VET. BRNO 1997, 66: 141-146

CIRCADIAN CHANGES IN ELECTROLYTE CONCENTRATIONS IN PLASMA AND ERYTHROCYTES IN TWO-WEEK-OLD CALVES

E. SKOTNICKA, W. F. SKRZYPCZAK, M. OZGO

Department of Animal Physiology, University of Agriculure, Szczecin, Poland

Received March 20, 1997
Accepted November 4, 1997

Abstract

Skotnicka E., W. F. Skrzypczak, M. Ozgo: *Circadian Changes in Electrolyte Concentrations in Plasma and Erythrocytes of Two-week-old Calves.* Acta vet. Brno 1997, 66: 141-146.

The aim of this study was to determine changes in the content of Na, K, Cl, Ca, Mg and P in the plasma and erythrocytes of 14-day–old calves with simultaneously performed evaluation of the occurrence and analysis of the rhythms.

The studies were carried out on a group of 10 Black and White, clinically healthy female calves aged 14 days. Seven days before and during the experiment, the animals were kept in uniform environmental conditions (LD = 16:8).

Blood samples were collected seven times over a period of 24 hours, spaced by 4 hour intervals, i. e., at 9:00, 13:00, 17:00, 21:00, 1:00, 5:00 and 9:00. Whole blood and plasma were used in the analysis. In the whole blood, hematocrit and concentrations of Na, K, Cl, Ca, Mg and P were determined. The plasma osmolality was also determined. The concentrations of sodium, potassium and chlorides in the erythrocytes were estimated using an indirect method.

In the present study, the circadian variations in plasma concentrations of sodium, potassium and chlorides and in its osmolality were found in two-week-old female calves. Changes in plasma content of sodium and potassium within twenty-four hours were significant ($P<0.01$). On the other hand, calcium, magnesium and phosphorus concentrations in plasma and concentrations of sodium, potassium and chlorides in erythrocytes were stable over the twenty-four hour period under study.

Female, calves, rhythms, plasma, erythrocytes, electrolytes, plasma osmolality

Biological rhythms are a manifestation of adaptation of living creatures to the environmental conditions, and enable them to maintain their homeostasis, increasing also chances for survival (Bünning 1967; Jilge 1993; Mick et al. 1994).

A stable level of mineral components in the blood testifies to efficiency of internal organs, above all to efficiency of kidneys which are the main regulator of isoinia and neurohormonal maturity of regulatory mechanisms (Koopman et al. 1985, Luke et al. 1990; Luke et al. 1991; McCaa 1992).

Functional immaturity of kidneys was observed in calves in the first days of life (Spitzer 1985; Skrzypczak 1991). The response of this organ to hormonal stimulation is also different than in adult animals, e. g. to aldosterone (Cugini et al. 1991; Safwete et al. 1992; Skrzypczak et al. 1994; Steele et al. 1994). It results from earlier studies that kidneys of newborn calves do not show functional changes within the circadian rhythm, thus indicating immaturity of the main mechanism enabling adaptation to varying environmental conditions over a twenty-four-hour period (Hellbrügge 1960; Wolf et al. 1991; Safwate et al. 1992; Weinert 1994). Circadian rhythms in the functions of calves' kidneys become visible about 10–14 day of life (Ketz 1960; Skrzypczak et al. 1992).

In connection with the above it is important, both from practical and applicable point of view, to find whether in calves which passed the second week of life there are changes in the blood level of main electrolytes over twenty-four-hour period and whether such changes influence internal stability of the blood cells (erythrocytes).

Materials and Methods

Animals

The studies were carried out on a group of 10 Black and White, clinically healthy female calves at the age of 14 days.

The animals were kept in individual boxes in uniform environmental conditions (LD=16:8) seven days before and during the studies. They were fed dams'milk (from a bucket), 8 l per day, two times a day, at about 6:30 and 17:30. They were also given free access to hay and water.

Before the experiment began, the external jugular vein of animals was catheterized to enable blood sampling without stress.

Analytical procedure

Blood samples were collected over a period of 24 hours, seven times at 4 hour intervals, i. e., at 9:00, 13:00, 17:00, 21:00, 1:00, 5:00 and 9:00. The blood was collected into heparinized tubes containing heparin (250 I. U. of Heparinum Polfa, Poland).

Whole blood and plasma were used in the analysis. To determine the content of studied components in erythrocytes, whole blood was hemolyzed adding 9 ml of deionized water to 1 ml of blood. To obtain the plasma, the remaining part of the blood was centrifuged. The plasma and the hemolyzate were stored at (–20) °C until analyses were done.

Biochemical analysis

In the whole blood hematocrit was determined, and concentrations of the following mineral components in the plasma (using the kits Analco, Poland):

- calcium (complexon method using o-cresolophtaleins);
- magnesium (complexon method using calmagit);
- inorganic phosphorus (molybdate method).

In the plasma and the hemolyzate, concentrations of following elements were measured:

- sodium and potassium (flame photometry method using a Flapho-40 photometer);
- chlorides (potentiometric titration method using a Spexon-100 chlorimeter).

The plasma osmolality was also established (cryoscopic method using a Knauer osmometer).

Concentration of sodium, potassium and chlorides in the erythrocytes were estimated using the indirect method based on the solution strength law, using hematocrit and the plasma and whole blood concentration of ions according to the following formula (Janiak 1989):

$$C_{er} = (100 \times C_{wb} - (100 - Ht) \times C_{pl})/Ht$$

where:

C_{er} = ion concentration in erythrocytes;
C_{pl} = ion concentration in plasma;
C_{wb} = ion concentration in whole blood;
Ht = hematocrit.

Statistical analysis

The results were statistically evoluated using analysis of variance and Duncan's D-test (Statgraphics v. 5.0 software). The Chronos package was used to establish the occurrence of the rhythm cycles and acrophases.

Results and Discussion

The results obtained in the experiment indicate the occurrence of circadian changes in concentrations of sodium and potassium in the plasma of two-week-old calves (Table 1).

The concentration of sodium and potassium in the plasma of calves showed circadian variability with an acrophase for sodium at 20:08 and an acrophase for potassium at 8:35. The plasma osmolality and plasma chloride concentrations changed over a 24-hour period as well, but these changes were not significant. Similar results were obtained by Kanabrocki et al. (1973). They observed, while studying the circadian changes in the mineral composition of human blood (at LD 15:9) that the acrophase for sodium ions occurred between 19:00 and 20:00, whereas for potassium ions between 23:00 and 24:00. The highest concentration of chlorides was found by them at about 22:00, that is at the beginning of the dark phase.

Table 1

Circadian changes of plasma Na, K, Cl, Ca, Mg and P concentrations and plasma osmolality in examined calves (n=10, x ± SD)

Hours of experiment	9:00 A	13:00 B	17:00 C	21:00 D	1:00 E	5:00 F	9:00 G
Na (mmol/l)	129.9 4.0	129.7 4.0	132.7 3.9 C→ABG*	135.2 3.6 D→ABCG	133.2 3.1 E→ABG	131.4 2.5 F→ABDEG	129.9 3.6
K (mmol/l)	5.30 0.42	5.26 0.44	4.82 0.38 C→ABG	4.50 0.40 D→ABCG	4.84 0.42 E→ABDG	5.18 0.38 F→ABCDE	5.30 0.33
Cl (mmol/l)	99.7 4.9	99.8 5.5	104.5 8.3	103.3 4.9	101.7 5.8	103.6 8.9	99.7 5.7
Ca (mmol/l)	2.69 0,.14	2.77 0.14	2.72 0.12	2.69 0.14	2.69 0.11	2.67 0.15	2.69 0.12
Mg (mmol/l)	0.74 0.05	0.72 0.07	0.71 0.08	0.71 0.07	0.75 0.06	0.72 0.06	0.74 0.05
P (mmol/l)	2.24 0.19	2.31 0.20	2.25 0.21	2.24 0.17	2.29 0.17	2.29 0.20	2.24 0.16
C osm (mmol/k H_2O)	289.0 6.7	286.4 6.6	283.5 8.8	286.2 7.5	288.7 5.9	283.8 7.6	289.0 6.2

Explanations: A, B, ..., G – significant differences (P<0.01)
*C→ABG – significant differences between Na concentration at 17:00 (C) and Na concentration at 9:00 (A), 13:00 (B), 9:00 (G).

The observed changes in the Na, K and Cl concentrations in the plasma of the studied calves were probably a result of the circadian periodicity in the function of kidneys (Aizman et al. 1983; Koopman et al. 1985; Wolf et al. 1991; Katinas et al. 1992; Safwate et al. 1992; Aizman et al. 1994). Distinct circadian changes in the activity of kidneys in young calves were observed by Ketz (1960), and by Skrzypczak et al. (1992). It is well-known that the activity of kidneys in the neonatal period shows great differences in comparison with the period of full somatic maturity. The blood flow through the kidneys is lower, as well as the level of glomerular filtration rate; the tubular resorption and secretion processes in tubules, particularly of water and electrolytes, are less efficient, and the excretability of excessive amounts of sodium and water is smaller (Dalton 1968; Skrzypczak 1989; Skrzypczak 1991; Safwate et al. 1992). The circadian rhythms in the activity of kidneys in calves begin to occur during the second week of the postnatal life in response to regular changes in the blood level of hormones controlling the function of kidneys and the activity of their receptors (Itoh et al. 1985; Robbillard et al. 1988; Ballauf et al. 1991; Nielsen et al. 1991; Wolf et al. 1991; Skrzypczak et al. 1992; Ruddy et al. 1993).

The findings of Baranow-Baranowski et al. (1980) indicate that a gradual increase in the activity of adrenal cortex occurs in the postnatal period. Similar results were obtained by Hartmann et al. (1987) and Spitzer (1982). These authors are of the opinion that a rapid increase in the activity of the adrenal cortex after birth is due to a low supply of electrolytes (mainly Na) and to their increased need during the organisms development process. Skrzypczak (1991) showed that the mineralocorticoid regulation of Na and K excretion is different in calves when compared to adult animals. Other authors also point out lower efficiency of the RAA system in calves (Hartmann et al. 1987; Skrzypczak 1991).

Szczepańska et al. (1967) believe that the circadian rhythm of diuresis is due to blood fluctuations of vasopressine during a 24-hour period. These authors found that the circadian rhythm of the AVP level does not depend on the motoric activity phase as it occurs in the case of aldosterone secretion. Attention is called to consistence of diuresis fluctuations and the level of ADH in the blood with the course of changes in the excretion of electrolytes controlled by separate mechanisms (Mc Caa 1992).

The increase in the excretion of sodium, potassium and chlorides is observed during the organism's active phase (Luke et al. 1991; Kemp et al. 1922; Ruddy et al. 1993; Boemke et al. 1994). It was shown, among other things, that the rhythm of potassium excretion in humans is consistent with the rhythm of glomerular filtration, the plasma flow through kidneys and the diuresis rhythm (Koopman et al. 1989).

Skrzypczak et al. (1992) in studies performed on two-week-old calves observed a higher resorption of sodium and chlorides in the activity period (day-light time), lower resorption during the night hours, and increased urine excretion of these electrolytes at night (despite the lowered glomerular filtration load). These authors state that high tubular resorption of potassium in the night hours contributes to a decrease in the urine excretion of this ion during that time of day.

The twelve-hours shift observed during the experiment in the phase of potassium rhythm in relation to the rhythm of sodium is confirmative for dependence between opposed tubular resorption of sodium and potassium.

In the studied calves, no circadian changes in the concentration of calcium, magnesium and phosphorus were observed, their plasma levels being stable (table 1). Markowitz et al. (1984), however, when studying changes in the Ca, Mg and P concentrations in the blood of children, observed their circadian periodicity. Szyszka et al. (1992) in studies on circadian variability in the plasma concentration of magnesium in humans found sinusoidal changes of concentration with an acrophase in the evening hours and its decrease in the morning hours. They explain the lowest concentration of magnesium at that time of day by the peak in activity of catecholamines, aldosterone and ACTH.

Table 2

Circadian changes of erythrocytes Na, K, Cl concentrations in examined calves (n=10, x± SD)

hours of experiment	9:00	13:00	17:00	21:00	1:00	5:00	9:00
Na (mmol/l)	33.98 3.11	34.02 3.25	33.58 3.15	34.24 3.38	34.72 3.27	34.62 3.90	33.98 2.97
K (mmol/l)	44.80 3.66	44.66 3.52	44.89 3.52	44.79 3.64	44.80 3.63	44.78 3.73	44.80 3.21
Cl (mmol/l)	30.24 3.18	30.14 3.06	28.42 2.91	29.72 3.21	29.94 3.18	29.67 3.90	30.24 2.54

In the present experiment, the concentrations of sodium, potassium and chlorides were stable in the erytrocytes, despite the circadian changes in their plasma (Table 2).

These results confirm the ability of red cells to preserve homeostasis of the cell interior and indicate that cellular membranes of the erythrocytes are in possession of a mechanism securing the integrity of intracellular environment with physiological fluctuations in the plasma levels of electrolytes.

In conclusion: 1. The circadian variations in the plasma concentration of sodium and potassium were found in two-week-old calves. These changes were significant ($P<0,01$). 2. No significant changes were found in the chloride, calcium, magnesium and phosphorus concentration in the plasma, plasma osmolality and erythrocytes sodium, potassium and chloride concentration. 3. Physiological changes in the plasma concentration of sodium and potassium do not affect the electrolyte homeostasis of erythrocytes.

Cirkadiánní změny koncentrace elektrolytů v plazmě a v erytrocytech u dvoutýdenních telat

Cílem této studie bylo stanovení změn v obsahu Na, K, Cl, Ca, Mg a P v plazmě a v erytrocytech u 14denních telat simultánně vyšetřovaných na výskyt a analýzu střídání prvků během dne. Studie byla provedena ve skupině 10 černých a bílých telat, klinicky zdravých, samičího pohlaví. Sedm dnů před pokusem a během něj byla telata chována ve stejných podmínkách životního prostředí (LD = 16:8). Vzorky krve byly odebrány sedmkrát během 24 hodin ve 4 hodinových intervalech, tj. v 9:00, 13:00, 17:00, 21:00, 1:00, 5:00, a v 9:00 hodin. Krev a plazma byly podrobeny analýze, byla změřena hodnota hematokritu a stanovena koncentrace Na, K, Cl, Ca, Mg a P včetně plazmatické osmolality. Koncentrace sodíku, draslíku a chloridů v erytrocytech byla odhadnuta použitím nepřímé metody. Změny obsahu sodíku a draslíku během 24 hodin byly významné ($P < 0,01$). Naproti tomu hodnoty vápníku, hořčíku a fosforu v plazmě i koncentrace sodíku, draslíku a chloridů v erytrocytech se v této studii během 24 hodin nezměnily.

Acknowledgements

The authors wish to thank DrSc. Janus K. for the technical help, 15 years of collaboration and stimulating discussions.

References

AIZMAN, R. I., VELIKANOVA, L. K. 1983: Renal and extrarenalexcretion of xenobiotes electrolytes, their stimulation during postnatal development. The development of renal functions and water-salt metabolism in ontogeny. Zbl. Pharm. Pharmakother. Labordiagn. **122**: 1299–1304

AIZMAN, R. I., RABINOWITZ, L., MAYER-HARNISCH C. 1994: Circadian rhythms and time course of adaptive sodium and potassium excretion in rats after nephrectomy. Am. J. Physiol. **266**: 454–462

BALLAUFF, A., RASCHER, W., TOLLE, H. G., WEMBER, T. MANZ, F. 1991: Circadian rhythms of urine osmolality and renal extrection rates of solutes influencing water metabolism in 21 healthy children. Miner. Electrolyte Metab. **17**: 377–382

BARANOW-BARANOWSKI, S., FRIEDRICH, M., PIKUŁA, R., PISAREWSKA, K. 1980: Badania nad zdolnością nerek cieląt do wydalania elektrolitów w pierwszych dwóch miesiącach życia. Zesz. Nauk. AR Szczecin, Zoot. **83**: 187–197

BOEMCKE, W., PALM, U., COREA, M., SEELINGER, E., REINHARDT, H. E. 1994: Endogenous variations and sodium intake-dependent conponents of diurnal sodium excretion patterns in dogs. J. Physiol. Lond. **476**: 547–552

BÜNNING, E.: The physiological clock. Longmans, Green and Co., London 1967

CUGINI, P., BATTISTI, P., PALMA, L., CAVALLINI, M., POZZILLI, P., SCIBILIA, G., LETIZIA, C., SEPE, M., CASSISI, A., MACHIARELLI, A. 1991: Circadian rhythm of the renin- angiotensin-aldosterone system in subject with kidney and heart transplants. Recenti Prog. Med. **82**: 4–8

DALTON, R. G. 1968: Renal function in neonatal calves-inulin, thiosulfate and PAH clearance. Br. Vet. J. **124**: 498–502

HARTMANN, H., SCHMIETENDORF, L., DEVAUX, S., FINSTERBUSCH, L., MAYER, H. 1987: Beziehungen zwischen Durchfallerkrankung und Nierenfunktion bei Kalb. Arch. Exp. Vet. Med. **41**:129–139
HELLBRÜGGE, T. 1960: The development of circadian rhythms in infants. Symp. Quant. Biol. **25**: 510–530
ITOH, N., MURAKAMI, D., NAITOH, Y., SATOH, R. 1985: Plasma aldosterone levels and plasma renin activity during the neonatal period in calves. Jap. J. Vet. Sci. **47**: 397–401
JANIAK, T. 1989: Diagnostyka kliniczna chorób wewnętrznych zwierząt domowych. PWN, Warszawa, 243.
JILGE, B. 1993: The ontogeny of circadian rhythms in the rabbit. J. Biol. Rhythms **8**: 247–261
KANABROCKI, E. L., SCHEVING, L. E., HALBERG, F., BREWER, R. L., BIRD, T. J. 1973: Circadian variations of biochemical blood profile in man. Space Life Sci. **4**: 258–265
KATINAS, G. S., RIABOV, S. I., KURUMSHIEVA, A. S. 1992: Circadian rhythm parameters of partial kidney functions in chronic glomerulonephritis. Klin. Med. Mosk. **70**: 106–109
KEMP, G. J., BLUMSOHN, A., MORRIS, B. W. 1992: Circadian changes in plasma phosphate concentration, urinary phosphate excretion, and cellular phosphate shifts. Clin. Chem. **38**: 400–402
KETZ, H. A. 1960: Untersuchungen zur Nierenfunktion und Renalen Elektrolytausscheidung beim Kalb und Rind. Arch. Exp. Vet. Med. **14**: 321–334
KOOPMAN, M. G., KREDIET, R. T., ARISZ, L. 1985: Circadian rhythms and the kidney. A review. Neth. J. Med. **28**: 416–423
KOOPMAN, M. G., KOOMEN, C. M., KREDIET, R. T., DE MOOR, E. A., HOEK, F. J., ARISZ, L. 1989: Circadian rhythm in glomerular filtration rate in normal individuals. Clin. Sci. **77**: 105–111
KOOPMAN, M. G., KOOMEN, G. C., van ACKER, B. A., ARISZ, L. 1994: Urinary sodium excretion in patients with nephrotic syndrome and its circadian variations. Quart. J. Med. **87**: 109–117
LUKE, D. R., WASEN, K. M., VADIEI, K. 1990: Lack of circadian variation in renal function in the obese rat: influence of continuous food and water intake. Prig. Clin. Biol. Res. **341**: 131–139
LUKE, D. R., WASEN, K. M., VADIEI, K. 1991: Circadian variation in renal function of the obese rat. Renal Physiol. Bioch. **14**: 71–80
MARKOVITZ, M. E., ROSEN, J. F., MIZRUCKI, M. 1984: Circadian rhythm of blood minerals during adolescence. Pediat. Res. **18**: 456–462
MC CAA, R. E. 1992: Rola of the renin-angiotensin-aldosterone and kallikrein-kinin systems in the control of fluid and electrolyte metabolism, renal function and arterial blood pressure. Clin. Exp. Hypertens. **4**: 1593–1598
MICK, G., JOUVET, M. 1994: Circadian rhythms – anatomical, functional and molecular bases. Med. Sci. **134**: 122–151
NIELSEN, H. K., LAURBERG, P., BRIXEN, K. MOSEKILDE, L. 1991: Relations between diurnal variation in serum osteocalcin, cortisol, parathyroid hormon and ionized calcium in normal individuals. Acta Endocrinol. **124**: 391–398
ROBILLARD, J. E., NAKAMURA, K. T. 1988: Neurohumonal regulation of renal function during development. Am. J. Physiol. **254** (Renal Fluid Electrol Physiol 23):777–779
RUDDY, M. C., AVORA, A., MALKA, E. S., BIALY, G. B. 1993: Blood pressure variability and urinary electrolyte excretion in normotensive adults. Am. J. Hypertens. **6**: 480–486
SAFWATE, A., DAVICCO, M. J., BARLET, J., DELOST, P. 1982: Sodium and potassium balances and plasma aldosterone levels in newborn calves. Reprod. Nutr. Develop. **22**: 689–696
SKRZYPCZAK, W. F. 1989: Kidneys functions of calves in the neonatal period. Part. I. Examination of the capability to save electrolytes Na, K, Cl, Ca, Mg, P. Pol. Arch. Wet. 29, 1–2, 23–33
SKRZYPCZAK, W. F. 1991: The values of water spaces and chosen parameters of renal function in the first week of calves postnatal life. Zesz. Nauk. AR Szczecin, Rozprawy Nr. 136
SKRZYPCZAK, W. F., JANKOWIAK, D., MUSZCZYŃSKI, Z., JANUS, K. 1992: Diurnal changes in renal function in calves. Part. I. Glomerular filtration rate., Part II. Electrolyte excretion. Mat. IX Kongres PTNW, Olsztyn, 452, 453
SKRZYPCZAK, W. F., JANUS, K., MUSZCZYŃSKI, Z., JANKOWIAK, D. 1994: The effect of spironolactone (Aldactone) on electrolyte balance and renal functions of calves. Acta vet. Brno **63**: 19–23
SPITZER, A. 1982: The role of the kidney in sodium homeostasis during maturation. Kidney Int. **21**: 539–544
STEELE, A., VEBER, H., QAGGIN, S. E., SCHEICH, A., ETHIER, J., HALPERIN, M. L. 1994: What is responsible for the diurnal variation in potassium excretion? Am. J. Physiol. **267**: 554–560
SZCZEPAŃSKA, E., PREIBISZ, J., DRZEWIECKI, K., KOZŁOWSKI, S. 1967: Dobowy rytm zmian poziomu hormonu antydiuretycznego we krwi u ludzi. Pol. Arch. Med. Wewn. **39**: 475–481
SZYSZKA, A., BROCKI, Z., MROZIEWICZ, A., PARADOWSKI, S. 1992: Dobowe zmiany stężenia magnezu w surowicy zdrowych ludzi. Pol. Tyg. Lek. **47**: 1078–1079
WEINERT, D. 1994: The development of circadian rhytmicity in neonates. Early. Hum. Dev. **36**: 117–130
WOLF, J. P., DAVICCO, M. J., GIRY, J., BARLET, J. P. 1991: Diurnal change in plasma natriuretic factor and arginine-vasopressin in young Jersey calves. J. Dev. Physiol. **6**: 363–366

Address for correspondence: Ewa Skotnicka, Department of Animal Physiology, University of Agriculture, Doktora Judyma St. No 6, 71-466 Szczecin, Poland. Phone: (048) 091 541521 ext. 340, fax: (048) 091 541642.