THE INFLUENCE OF SLEEP DEPRIVATION ON LEVELS OF SERUM HORMONES IN HEALTHY CALVES
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Abstract

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The influence of 24 h of sleep deprivation on the blood serum concentration of aldosterone, thyrotropine, triiodothyronine, and thyroxine was studied in healthy calves. In the experiment, 14 calves (Bohemian Pied x Holstein) aged 3 months with mean body mass of 85 kg were used. Blood samples were collected from the v. jugularis externa. The concentration of hormones was measured using RIA.

A sleep deprivation of 24 h led to a statistically significant (P<0.05) increase of aldosterone and thyrotropine levels and to a significant (P<0.05) decrease of triiodothyronine. The level of thyroxine and the triiodothyronine/thyroxine ratio were not changed significantly.

Calf, stress, aldosterone, TSH, T₃ and T₄.

The loss of afferent nervous stimulation from mechanoreceptors of the heart ventricles as a consequence of the implantation of a total artificial heart leads to changes of both neural as well as humoral feedback control (I m a c h i et al. 1983; V a š k ů et al. 1987). Changes have been described in the levels of thyroid hormones, aldosterone, renin, catecholamines, and vasopressin (B ü c h e r l et al. 1979; I m a c h i et al. 1983; S t a n l e y et al. 1976; V a š k ů et al. 1987; W e b s t e r et al. 1978). Both the specific effects of the total artificial heart as well as non-specific stress reaction play a role in the changes of some hormone levels (S t a n l e y et al. 1976; V a š k ů 1965; W e b s t e r et al. 1976). To determine the influence of the latter factor this preliminary study undertook an investigation of the influence of a 24-h sleep deprivation on the serum level of selected hormones. Another aim of this study was to determine physiological values of thyroid gland hormones in calves used in total artificial heart experiments.
Materials and Methods

The blood for measurement of aldosterone, thyrotropine - TSH, triiodothyronine - T₃ and thyroxine - T₄ concentration was taken from 14 clinically healthy calves (6 females, 8 males). The animals (Bohemian Pied x Holstein) had a mean body mass of 85 kg; their age was 3 months. The calves were restrained individually in stalls of a common calf-shed. The temperature in the barn at the time of sampling was 25 °C; blood samples were collected between 10-11 a.m. daily, i.e. 2-3 h after feeding. The first sampling was the control, the second collected 24 h after sleep deprivation, was the experimental.

Sleep deprivation was induced by causing the calves to get up each hour. Blood was drawn by aspiration from a direct venipuncture of the v. jugularis externa, using a needle and a plastic syringe. The blood was transferred into glass test tubes and centrifuged at 2,500 r.p.m. for 20 minutes. The serum samples were placed into glass test tubes and stored at -18 °C pending analysis (within 6 weeks).

Aldosterone concentration was measured using methods described in our previous study (Dostál et al. 1985). Thyroxine (T₄), triiodothyronine (T₃) and thyrotropine (TSH) were measured using classical RIA methods modified for laboratory requirements of the Department of Nuclear Medicine, the District Institute of National Health at Znojmo. The TSH concentration was determined using a specific antibody Calbiochem - Boehringer Lot No. 142107; the hormone was labelled by chloramin iodation using Na⁺I²⁻ made by Amersham (England) The separation of a binding fraction was carried out using another antibody - Riagar. The sensitivity of the method for TSH measurement was 1.25 mU.l⁻¹, variation coefficient 13.5 %.

The T₃ determination was carried out using a specific antibody from the Research Institute of Endocrinology at Lubochňa, radioligand from ŠúrvJT Košiče. Another antibody - Riagar from Bioveta Ivanovice na Hané - was used for separation of the binding fraction. The sensitivity of the method was 0.2 nmol.l⁻¹, variation coefficient 9.2 %.

To measure the T₄ concentration, a specific antibody was employed as provided by the Institute of Experimental Endocrinology, Slovak Academy of Science in Bratislava, commercial radioligand from ŠúrvJT Košiče: the separation of a binding fraction was carried out with polyethylene glycol Mr 6,000. The sensitivity of the method was 3.4 nmol.l⁻¹, variation coefficient 6.8 %.

The T₃/T₄ ratio was calculated as the ratio of T₃ and T₄ levels in the plasma in nmol.l⁻¹ and multiplied by 10³ as reported by Schellenger et al.(1984).

The means and standard deviations of the values were calculated for each experimental animal. Student's t-test for analysis of means was used for comparison of pairs. The P values of less than 0.05 were considered significant (Reisenauer 1970).

Results

The mean values, standard deviations, and standard errors of the means for the hormones studied, before and after 24 h of sleep deprivation are presented in Table 1. After sleep deprivation, aldosterone and TSH were elevated by 184 % and 22 %, respectively. The concentration of T₃ was
Table 1
Serum hormones levels before and after 24 hrs sleep deprivation (n = 14)

| quantity          | before  | after  | t-test  |
|-------------------|---------|--------|---------|
| ALDO (pmol/l)     | $\bar{x}$ | 33.26  | 94.35   | $\pm$ S.D. | 20.69  | 63.55   | P < 0.05 |
|                   | $\pm$ SEM | 5.53   | 16.98   |          |
| TSH (mU/l)        | $\bar{x}$ | 2.31   | 2.81    | $\pm$ S.D. | 0.57   | 0.63    | P < 0.05 |
|                   | $\pm$ SEM | 0.15   | 0.17    |          |
| $T_3$ (nmol/l)    | $\bar{x}$ | 1.99   | 1.68    | $\pm$ S.D. | 0.35   | 0.34    | P < 0.05 |
|                   | $\pm$ SEM | 0.09   | 0.09    |          |
| $T_4$ (nmol/l)    | $\bar{x}$ | 109.56 | 104.16  | $\pm$ S.D. | 29.74  | 20.39   | N.S.    |
|                   | $\pm$ SEM | 7.95   | 5.45    |          |
| S-T$_3$/S-T$_4$ x 10$^3$ | $\bar{x}$ | 18.1   | 16.1    | $\pm$ S.D. | 1.8    | 6.6     | N.S.    |
|                   | $\pm$ SEM | 1.3    | 6.5     |          |

decreased by 16 %, that of $T_4$ by 5 % and their ratio ($T_3/T_4$) was decreased by 11 %. The increase of aldosterone and TSH levels and the decrease of $T_3$ were significant (P<0.05) with regard to controls (Fig. 1).

Discussion

The results obtained from control and experimental samples are within the physiological values of these hormones which have been reported for man: aldosterone up to 400 pmol.l$^{-1}$ in a resting position after a few hours rest), TSH up to 5 mU 1$^{-1}$, $T_3$ 1.5 - 3.5 nmol l$^{-1}$, $T_4$ 60 - 160 nmol 1.$^{-1}$. The
Fig. 1. Serum aldosterone (pmol l\(^{-1}\)), thyrotropine (TSH, mU l\(^{-1}\)) and triiodothyronine (nmol l\(^{-1}\)) levels before and after sleep deprivation. The values are presented as means ± S.E.M.; * = P < 0.05.
ratio of $T_3/T_4$ is reported to be within a range of 12-32 in adult healthy persons (both men and women) (Erfurth and Hedner 1986).

The levels of aldosterone were significantly decreased in the present group of calves when compared with our previous observations. In calves of the same age, body mass, confinement and feeding procedures, employed in a previous study, mean values of aldosterone of $85.6 - 26.9 \text{ pmol.l}^{-1}$ (Dostal et al. 1985). However, that experiment was conducted in a different season (January 1984), whereas the present study was carried out in August 1986. These differences show the importance to consider the influence of season on plasma hormone concentrations.

Increased levels of aldosterone after 24-hours sleep deprivation can be explained by a neurosecretory response of the hypothalamus. After the release of corticotropin-releasing hormone (corticoliberin) an increase of adrenocorticotropic hormone (ACTH) occurs which stimulates directly the cells of the zona glomerulosa of the adrenal gland, resulting in an increase in the plasma level of aldosterone (Burchnfield 1985; Vaszk 1965).

The data of the TSH levels as a sequel to stress are in conflict. Reports of its increased secretion are few; most describe it as being inhibited (Schreiber 1985). The differing data may reflect differences in the experimental design, especially the intensity of stress, stimulus and the reaction of the individual or the species.

Even the data of the response of the thyroid hormones ($T_3$, $T_4$) on acute stress are also differing. Permanent physical stress connected with insufficient dietary energy intake leads to the drop of both $T_3$ and $T_4$ with simultaneous shift to the deiodination of thyroxine in tissues (Schreiber 1985). Sleep deprivation of our experimental animals was also connected with greater physical activity (longer periods of standing, moving), while the amount of food offered was not changed.

The slight increase of TSH and decrease of $T_3$ and $T_4$ found in the present study is in agreement with data published of these levels in human patients suffering burn
injury. The level of T3 was decreased substantially more than that of T4. Our study recorded a non-significant decrease was caused by the decrease of T3 and T4 or it is a response to stimulating influence of thyroid releasing hormone (TRH), released from the hypothalamus in response to stress reaction (Burchfield 1985; Schreib 1985).

Regarding hormones of the thyroid gland and TSH, no data for calves of identical age and body mass category have been found in the available literature. Therefore, present data may serve as reference values for further research of hormones of animals with total artificial heart implanted and surviving for a long time. Further experimentation is needed of the link between stress and thyroid gland function.

Vliv spánkové deprivace na koncentrace hormonů v krevním séru zdravých telat (metodické sdělení)

Byl sledován vliv 24hodinové spánkové deprivace na hladiny některých hormonů v krevním séru (aldosteron, tyrotropin, trijodtyronin, tyroxin). Studie byla provedena u 14 te­lat (české strakaté x Holsteinské, věk 3 měsíce, průměrná hmotnost 85 kg), která byla ustájena ve společném teletníku. Krev pro stanovení hormonů byla odebírána punkcí v. jugu­laris externa. Koncentrace hormonů byly stanovovány RIA metodami.

Bylo zjištěno, že 24hodinová spánková deprivace vede ke statisticky významnému zvýšení hladin aldosteronu a ty­rotropinu a ke snížení trijodtyroninu. Hladina tyroxinu a poměr trijodtyronin/tyroxin se významně neměnily.

Влияние депривации сна на уровне гормонов в кровяной сыворотке здоровых телят (методическое сообщение)

Авторы изучали влияние суточной депривации сна на уровне некоторых гормонов в кровяной сыворотке
(альдостерон, тиротропин, трийодтиронин, тироксин). Исследование проводили у 14 телят чешской пестрой породы, скрещенной с гольштейнской породой (в возраст 3 месяца, средняя масса 85 кг), содержащихся в совместном теляннике. Взятие крови для определения гормонов проводили пункцией наружной яремной вены. Уровни гормонов определяли методами РИА.

Было установлено, что суточная депривация сна приводит к статистически значимому повышению уровня альдостерона и тиротропина и понижению трийодтиронина. Уровень тироксина и соотношение трийодтиронин/тироксин существенно не менялись.

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