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Circadian Rhythm of Cortisol Secretion in Dogs of Different Daily Activities

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Abstract

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The concentrations of immunoreactive cortisol were monitored over 24 hours in the serum of a total of 23 dogs. The dogs were divided into three groups: (a) control group with no exercise, (b) experimental group, (c) working dogs. Blood was collected from each dog at the intervals of 90–180 min. The cortisol concentrations in different time periods were mutually compared using the Kruskal-Wallis test for the analysis of variance. Circadian pattern was found in the study. Cortisol concentrations found between 10.00 and 13.00 (46.5 ± 17.0 nmol/l) were significantly higher ($P < 0.05$) than those found between 19.00 and 22.00 (38.9 ± 16.0 nmol/l). The results differed within individual groups. Diurnal rhythms were confirmed in control group but not found in experimental group and in working dogs. In experimental group, other significant ($P < 0.05$) differences were also found between various time intervals compared to the total pattern but no diurnal rhythm was established. In working dogs no significant differences ($P < 0.05$) were found between cortisol concentrations in different time intervals. The results suggest that cortisol concentrations in the dog are subject to considerable changes during the day. Depending on the characteristics of day and night activities a diurnal rhythm can be found in some dogs.

Diurnal rhythm, cortisol concentration, 24 hours, exercise

The activity of adrenal cortex shows clear circadian pattern in humans (Pincus 1943). Cortisol concentrations are highest between 04.00 and 08.00 h, decrease during the daytime and reach its minimum between 20.00 h and midnight (Kreze 1992). The adrenal gland does not release cortisol into blood continuously but in short pulses. Between the pulses the secretion practically ceases (Weitzman et al. 1971). Rhythmic variations of cortisol secretion are also reflected in the excretion of unconjugated cortisol in urine (Pinsker and Bultasová 1979). The circadian pattern of cortisol secretion is firmly fixed in the man and does not change even during most of chronic conditions (Pinsker and Bultasová 1979). Exceptions can be found in cases of some pathological conditions affecting the hypothalamus, and/or impairing consciousness or sleep (Krieger and Krieger 1966). Circadian rhythm of cortisol secretion is affected in most patients with Cushing's syndrome. In psychologically unstable persons the evaluation of circadian rhythm of cortisol secretion is difficult, in particular if such persons are exposed to stress for instance by frequent blood sampling (Pinsker and Bultasová 1979).

Cortisol concentrations in dogs during the day were studied for the first time by Rijnberk et al. (1968), and by Johnston and Mather (1978). Rijnberk et al. (1968) collected blood from dogs every three hours and observed the differences in concentrations of 11β-hydroxycorticosteroids (11β-OHCS) over 24 h. In six out of eight dogs included in the study they described a diurnal rhythm of secretion of 11β-hydroxycorticosteroids similar to the situation in humans. However, the differences found were less distinct than in humans. Other

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authors who dealt with the same topic did not confirm the circadian cortisol secretion rhythm. Takahashi et al. (1981) collected blood from dogs every 30 min over the period of 28 h and detected no circadian rhythm. Kemppainen and Sartin (1984) also studied circadian rhythm of cortisol secretion. They collected blood from nine healthy dogs and two other dogs after adrenalectomy. Blood was collected at 20 min intervals over a total period of 25 h. Neither these authors confirmed circadian rhythm of cortisol secretion. Palazzolo and Quadri (1987) described circadian rhythm of cortisol secretion in adult animals only, while in puppies and old dogs no rhythmic variations in cortisol secretion were found during the day. In two dogs out of four in their study, Van Vonderen et al. (1997) found an almost two-fold greater urine cortisol/creatinine ratio in the samples collected during the day compared to those collected during the night.

The objective of the present work was to monitor the circadian rhythm of cortisol secretion over a period of 24 h in dogs with different activities: (1) normal way of life without significant physical and psychical load, and (2) exposed to long-term physical and psychological load (experimental dogs and working dogs).

Materials and Methods

Dogs, daily rhythm, exercise and load

In total 23 dogs were included in the study. The dogs were divided in to three groups: (1) dogs with common daily routine with no particular physical and emotional load (n = 5), (2) experimental dogs (beagles) used for experimental purposes on a long-term basis (n = 7), (3) working dogs used for various operations (n = 11). The experimental groups contained only individuals with no signs of any disease detectable either clinically or by laboratory examination (haematological and biochemical examination of blood) and with the history of no serious condition in the past. The groups consisted of the individuals of both sexes. The age of the dogs varied between one and 8 years. Regular daily activities were different among the groups. The first group consisted of males and females kept in kennels with usual activities during the day (walking, playing, contact with other dogs and people). Since their early age the dogs were used to contact with wide range of different people (students, staff of the clinic). The animals were resting during the night having no access to the run. There were 3 bitches and 2 dogs in the group. Two animals in the group were German shorthaired pointers and the remaining three were crosses of larger breeds. The animals in this group were regularly fed around 06.00 h. The second group also consisted of dogs kept in kennels. During the day the dogs showed regular activity, during the night the dogs were at rest. Besides the regular daily activities the dogs in this group were also used for experimental purposes (blood sampling), which took place especially during morning hours. After 15.00 h the dogs were left undisturbed. The dogs in this group were not used to contact with wide range of people (except for the staff taking care of them and the veterinarian). All animals in this group were beagles, two of them were males and five females. Feeding was also regular around 14.00 h. The third group consisted of working dogs of the Ministry of Interior of the Czech Republic. This group consisted of 11 German shepherds – 6 females and 5 males. Some of these dogs were also kept in kennels, some stayed with the handlers at their homes. The daily routine in this group was rather variable. The dogs were used according to the needs of the police sometimes during the day and sometimes during the night. The police actions required fulfilment of patrolling, defence and trailing tasks. The animals in this group were fed individually at different times of the day. All working dogs in the third group were used to regular contact with wide range of people. All dogs in all three groups were used to undergo common veterinary procedures on a regular basis.

Blood sampling and analysis of hormones

Blood was collected from all dogs included in the experiment every 90–180 min over the period of 24 h. In the first and the second group the sampling was carried out at rest, in the working dogs the sampling was carried out during the periods of light load (training of defence and trailing tasks). In all groups the sampling took place in winter (December). During the sampling the dogs of groups 1 and 2 were housed at the ambient temperature around 20 °C. The dogs of the third group were outdoors between the sampling. The ambient temperature ranged between 0 °C and 10 °C. Blood was collected from *vena jugularis* and *vena cephalica antebrachii* by means of separate collections using a needle with a diameter of 0.7 mm. Permanent catheters were not introduced since regular daily and nocturnal activities of the dogs would be significantly impaired, which was impossible especially in the working dogs.

Serum was obtained from each blood sample through a standard procedure and was immediately frozen at -20 °C. Serum cortisol concentrations were determined by radioimmunoassay kit (Cortisol No. 1841 of Immunotech) according to the instructions of the manufacturer. The lowest detectable amount of cortisol (sensitivity of the method) was 10 nmol/l. Cross-reactivity (specificity of the method) with regard to other endogenous steroids was as follows: 11-desoxycortisol 18%, corticosterone 8.4%, 21-desoxycortisol, desoxycorticosterone 7.3%, 17α-hydroxyprogesterone 3.5%, dihydrocortisol 2.4%, 5α-dihydrocortisone 2.3%, progesterone 1.8%, cortisone 1.5%, pregnenolone 1.1%. Cross-reactivity with other endogenous steroids was less than 1%.

Statistical evaluation

Unistat software, version 5.1, was used for statistical evaluation of the results. The Kruskal-Wallis single-factor analysis of variance and graphical presentation of the results were used to evaluate the rhythmical pattern of cortisol secretion in different time intervals.

The experiments were carried out conform to the provisions of the Act on Protection of Animals against Cruelty No. 246/1992 as amended.

Results

Cortisol concentrations were compared within the whole group (all individuals) in different time intervals over 24 h using Kruskal-Wallis single-factor analysis of variance. Significant differences ($P < 0.05$) were found between cortisol concentrations in the periods of 10.00 to 13.00 h vs 19.00 to 22.00 h (Fig. 1). Cortisol concentrations during the period of 10.00 to 13.00 h (46.51 ± 17.02 nmol/l) were significantly higher ($P < 0.05$) than those found between 19.00 and 22.00 h (38.94 ± 16.09 nmol/l).

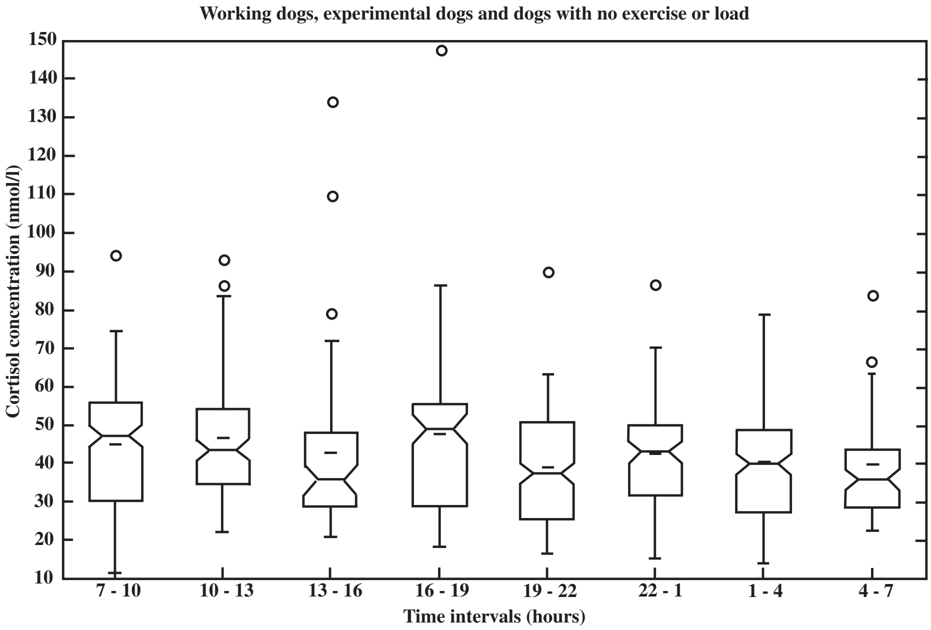

Fig. 1. Cortisol concentration – time intervals

The comparison of cortisol concentrations found within individual groups in different time intervals produced the results described below. In the dogs with no load (Fig. 4) highly significant differences ($P < 0.01$) were found between cortisol concentrations detected during the intervals of 10.00 to 13.00 h (40.1 ± 9.6 nmol/l) and those found between 16.00 and 19.00 h (23.0 ± 3.7 nmol/l). Furthermore differences were also found between cortisol concentrations detected during the intervals of 10.00 to 13.00 h (40.1 ± 9.6 nmol/l) and those found between 19.00 and 22.00 h (23.2 ± 5.9 nmol/l). Significant differences ($P < 0.05$) in this group of dogs were confirmed between cortisol concentrations in the following time intervals: 10.00 to 13.00 h (40.1 ± 9.6 nmol/l) versus 22.00 to 01.00 h (25.7 ± 8.6 nmol/l), and 10.00 to 13.00 h (40.18 ± 9.6 nmol/l) versus 13.00 to 16.00 h (26.1 ± 3.3 nmol/l).

In the group of experimental dogs (Fig. 3) significant differences ($P < 0.05$) were found between cortisol concentrations in the time intervals of 07.00 to 22.00 h (52.3 ± 8.3 nmol/l) and 13.00 to 16.00 h (48.0 ± 27.6 nmol/l). Furthermore differences were also found between cortisol concentrations detected during the following intervals: 13.00 to 16.00 h (48.0 ± 27.6 nmol/l) versus 16.00 to 19.00 h (53.2 ± 11.8 nmol/l), 04.00 to 07.00 h (44.3 ± 16.7 nmol/l) versus 07.00 to 10.00 h (53.9 ± 17.3 nmol/l), and 04.00 to 07.00 h (44.3 ± 16.7 nmol/l) versus 16.00 to 19.00 h (53.2 ± 11.8 nmol/l).

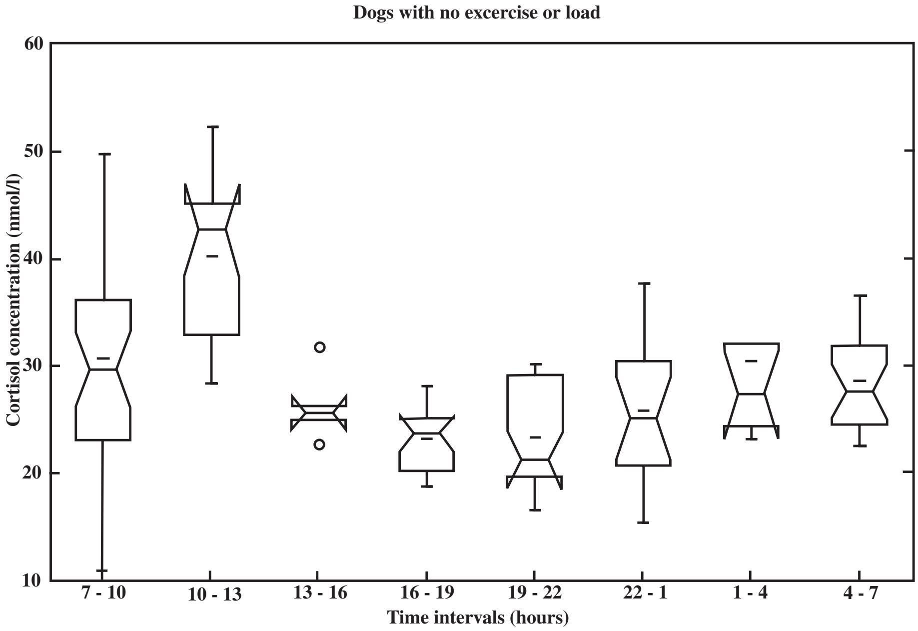

Fig. 4. Cortisol concentration – time intervals

In the group of working dogs (Fig. 2) no significant differences were found among cortisol concentrations in different time periods.

Discussion

The results of the present work showed that cortisol concentrations in dogs considerably vary during the day. Within the framework of general comparison among all individuals a diurnal pattern was found. Statistically significant differences were demonstrated between cortisol concentrations found during the intervals of 10.00 to 13.00 h versus 19.00 to 22.00 h in the group of all animals in the experiment. The results differed within individual groups. In the group of dogs with no load we demonstrated highly significant differences between cortisol concentrations in morning hours compared to late afternoons and evenings. In the group of experimental dogs we demonstrated in addition also significant differences among other time intervals practically during the whole period of observation. In the group of working dogs no significant differences in cortisol concentrations were found.

The differences can be explained as follows:

A diurnal activity was demonstrated by total assessment of cortisol concentrations in different time periods in the whole group, as well as in the dogs of group 1. Compared to the diurnal activity in humans, the pattern found in our study is less obvious (Pinsker and

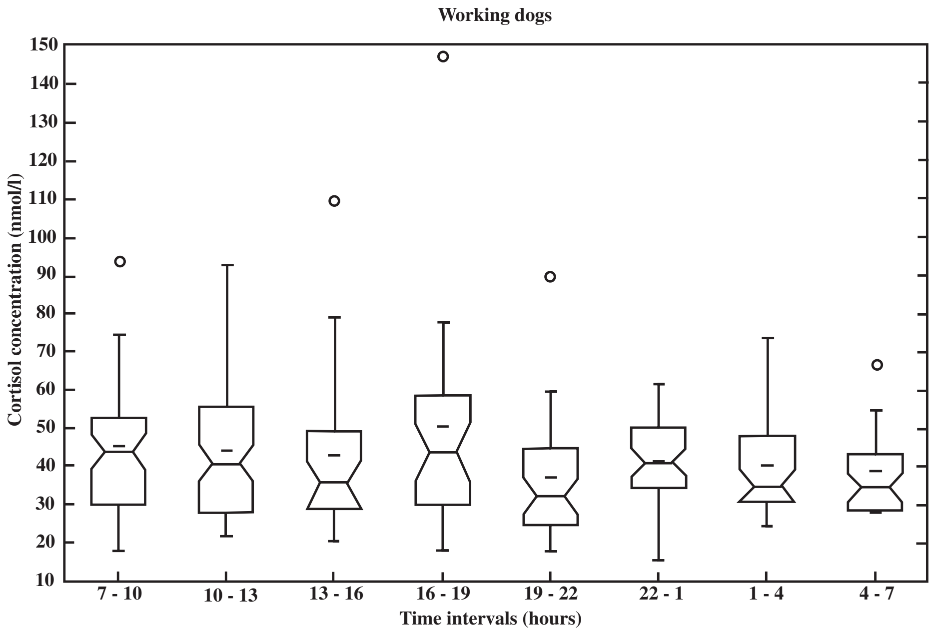

Fig. 2. Cortisol concentration – time intervals

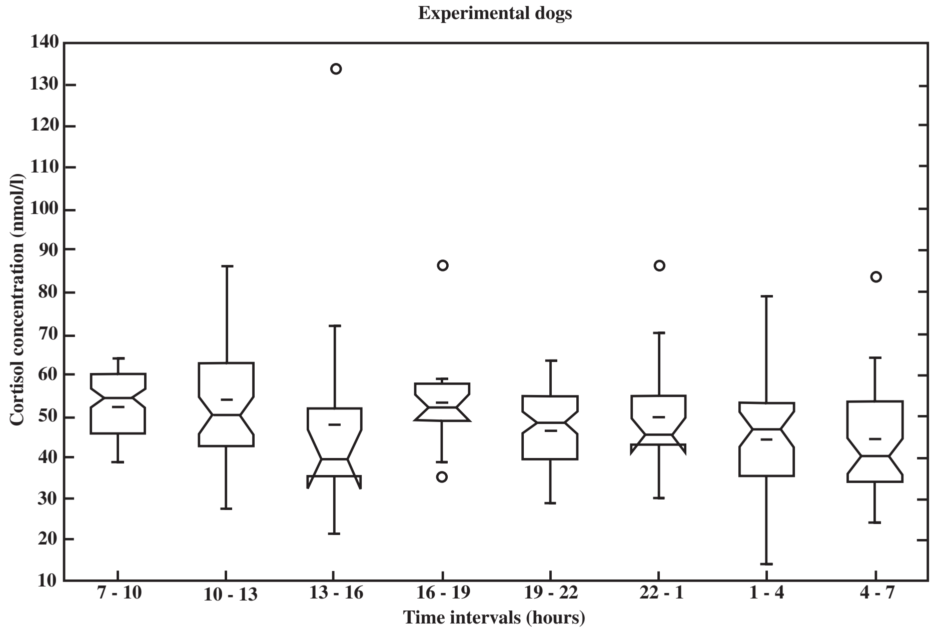

Fig. 3. Cortisol concentration – time intervals

Bultasová 1979; Halberg 1980). This can be due to several reasons. The activation of the hypothalamus-pituitary-adrenal axis depends on various external and internal factors and may differ in various species, breeds, both sexes and different individuals. Cortisol is released into blood in secretory waves (ultradian secretion pattern, Kooistra et al. 1997), which directly correlate with the release of ACTH from the anterior pituitary gland. The number of secretory pulses in the dog was reported to be around 10 in 24 h (Kemppainen and Sartin 1984). There is no significant difference with regard to the situation in humans with 5 to 10 pulses in 24 h reported by Krieger et al. (1971) or 7 to 13 pulses in 24 h as reported by Weitzman et al. (1971). While the distribution of secretory pulses of ACTH in humans is irregular with maximum in morning hours and minimum late in the evening (Krieger et al. 1971), in the dog the secretory pulses occur in a relatively regular pattern over the whole period of 24 h (Kemppainen and Sartin 1984; Orth et al. 1988). Various stress stimulations can also participate in the release of ACTH. Daily rhythm in the dog and in the man is rather different, in particular as regards the number of sleep episodes per day. It is well known that dogs sleep in more episodes (Takahashi et al. 1981). This fact can explain a possible lack of differences in circadian rhythm (compared to humans) or its total absence. The results of this work support such explanation. A well-expressed diurnal activity was found in the dogs of group 1, kept in kennels and exposed to regular daily load. The dogs in this group had normally contact with people during the day and rest during the night (with no access to outdoor runs).

In the dogs of group 2 (beagles kept for experimental purposes), rather variable results were found. Significant variations in cortisol concentrations were found among many time intervals during the day. No diurnal secretion pattern was apparent in this group. This can be explained by the fact that a regular daily rhythm was disturbed in the dogs by the procedures relating to our study (in particular as regards the periods of sleep and activity). In addition the dogs were exposed to more stress (frequent blood sampling by strange persons, lack of usual daily activities). Individual results therefore probably reflected a series of cortisol secretion peaks.

In the dogs of the working group no significant differences were detected among cortisol concentrations in different time periods. There were minimum variations during the day and night. These dogs were adapted over long time to a totally atypical daily rhythm (frequent activities during both daytime and night). We would explain the results by total lack of diurnal rhythm in cortisol secretion in these dogs.

In conclusion it is possible to summarise that cortisol secretion considerably varies during the period of 24 h. The variations depend on the activation of hypothalamus-pituitary-adrenal axis and is subject to numerous factors (individual patterns, breed, living conditions, characteristics and especially distribution of physical activity, rest and sleep during the day and night). These factors are far more important in dogs than in humans. Therefore in the dog the diurnal activity cannot be assessed in individual animals but only in groups kept and handled in different conditions and having different daily rhythm. This is in many cases certainly very difficult to arrange. Our results have shown diurnal rhythms in some dogs but failed to find them in others. In general, in the dog as animal species, however, the existence of diurnal pattern of cortisol secretion should be admitted, although the differences found are not as obvious as in humans.

Sledování cirkadiánní rytmicity sekrece kortizolu u psů s rozdílným denním režimem

Koncentrace imunoreaktivního kortizolu byla sledována v průběhu 24 hodin v séru celkem u 23 psů. Psi byli rozděleni do tří skupin: (a) kontrolní - bez zátěže, (b) experimentální a (c) služební. Krev byla u každého psa odebírána v 90–180 min

intervalech. Koncentrace kortizolu v jednotlivých časových úsecích byly mezi sebou porovnány ANOVA analýzou Kruskal-Wallisovým testem. V rámci všech sledovaných jedinců vykazovaly změny cirkadiánní charakter, mezi 10:00-13:00 hod (46,5 ± 17,0 nmol/l) byly koncentrace kortizolu statisticky významně vyšší (P < 0,05) než mezi 19:00-22:00 hod (38,9 ± 16,0 nmol/l). V rámci jednotlivých podskupin se výsledky lišily. Diurnální rytmicita byla prokázána u psů kontrolních a nebyla zjištěna u psů experimentálních a služebních. U skupiny experimentálních psů byly zjištěny statisticky významné rozdíly (P < 0,05) i mezi dalšími časovými intervaly než v rámci celkového srovnání, avšak nevykazovaly diurnální rytmicitu. U skupiny psů služebních nebyly statisticky významné rozdíly (P < 0,05) mezi koncentracemi kortizolu v jednotlivých časových intervalech zjištěny. Z výsledků práce vyplývá, že koncentrace kortizolu u psa během dne významně kolísá a v závislosti na charakteru denní a noční aktivity vykazuje u některých psů diurnální rytmus.

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