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PLASMA CORTISOL IN THE HORSE, DIURNAL RHYTHM AND EFFECTS OF EXOGENOUS ACTH

By

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LARSSON, M., L.-E. EDQVIST, L. EKMAN and S. PERSSON: *Plasma cortisol in the horse, diurnal rhythm and effects of exogenous ACTH.* Acta vet. scand. 1979, *20*, 16—24. — Peripheral blood plasma cortisol concentration and its diurnal variation was measured in 4 horses. Mean concentration of cortisol during 24 hrs. was 42 ng/ml (s ± 20 ng/ml). Peak values occurred at 6 a.m. and the lowest values were observed at about 6 p.m. (mean 65 ng/ml and 20 ng/ml, respectively).

Long-acting ACTH at a dose of 150 i.u. was given by intramuscular injection to the 4 horses. Peak cortisol concentrations markedly exceeding the prestimulation level were obtained between 2 and 4 hrs. after injection. During the immediate 24 hrs. after these peaks, the mean cortisol level was markedly lower and the cyclic variation out of phase with the basal diurnal pattern. After a gradual adjustment during the second postinjection day, no differences could be seen between the 2 patterns on day 3 after injection.

horse; cortisol levels; diurnal rhythm; ACTH.

The use of anti-inflammatory drugs, such as synthetic corticosteroids and ACTH, is widespread in equine practice. Untoward effects of the therapeutic use of these drugs have been pointed out, but little information is available on the duration of the effects of long-acting ACTH compounds and the pattern of the resulting corticoid response. In Sweden the time limit for treatment before racing is set to 96 hrs. for most drugs. Exceptions from this rule are made for e.g. anabolic steroids and glucocorticoids, for which the time limit is 14 days. It has been discussed whether long-acting ACTH should belong to this latter group or not.

The aim of the present study was to determine the normal plasma concentration of cortisol, its diurnal variation and the

intraindividual variation caused by frequent blood sampling, as well as the influence of exogenous long-acting ACTH on plasma cortisol levels and patterns.

MATERIALS AND METHODS

Four clinically sound standardbred geldings, 6—13 years of age, were used in the experiment. Samples of blood were collected into heparinized tubes by jugular venipuncture. After centrifugation the blood plasma was stored at below —18°C until assay. Initially the normal concentration of cortisol and its diurnal variation were determined in the 4 horses during a 24 hr. period by blood sampling at 4 hr. intervals. To study the role of frequent blood sampling on blood plasma cortisol content, 1 horse was sampled every 5 min. during 30 min. starting at 7.30 a.m.

The effects of exogenous ACTH on blood plasma cortisol concentration were studied in the 4 horses. Each horse received an intramuscular injection of 150 i.u. of a long-acting ACTH preparation (Acton Prolongatum® 60 i.u./ml, Ferring AB, Malmö, Sweden). Blood samples were obtained immediately before injection of ACTH and ¼, ½, ¾, 1, 1½, 2, 3, 4, 5, 6, 8, 11 and 13 hrs. after administration. Thereafter blood sampling continued for the following 48 hr. period with bleeding intervals of 3 hrs. and finally, during the following 60 hr. period, blood samples were obtained every 6th hr.

At a separate occasion 1 horse was injected i.m. with 300 i.u. of the same ACTH preparation and sampled at ½, 1, 1½, 2, 2½, 3, 4, 5, 9, 24, 48, 72, 96 and 120 hrs. after injection.

Cortisol levels were determined by a competitive protein binding technique, utilizing equine corticosteroid binding globulin and tritium-labelled cortisol (*Richkind & Edqvist* 1973). Blood plasma cortisol was extracted with dichloromethane. Separation of free and protein-bound hormone was performed by adsorbing the free fraction to magnesium silicate (Florisil). Protein-bound labelled cortisol was quantitated in a liquid scintillation spectrometer.

The precision of the assay was calculated from the differences between the duplicate determinations according to the formula

$$s = \sqrt{\frac{d^2}{2n}}$$

where s = standard deviation, d = differences between duplicates and n = number of duplicates. The coefficient of variation ranged between 6.4 % and 8.4 % at plasma cortisol levels ranging from 3 to 152 ng/ml. All values reported are means of duplicate determinations.

RESULTS

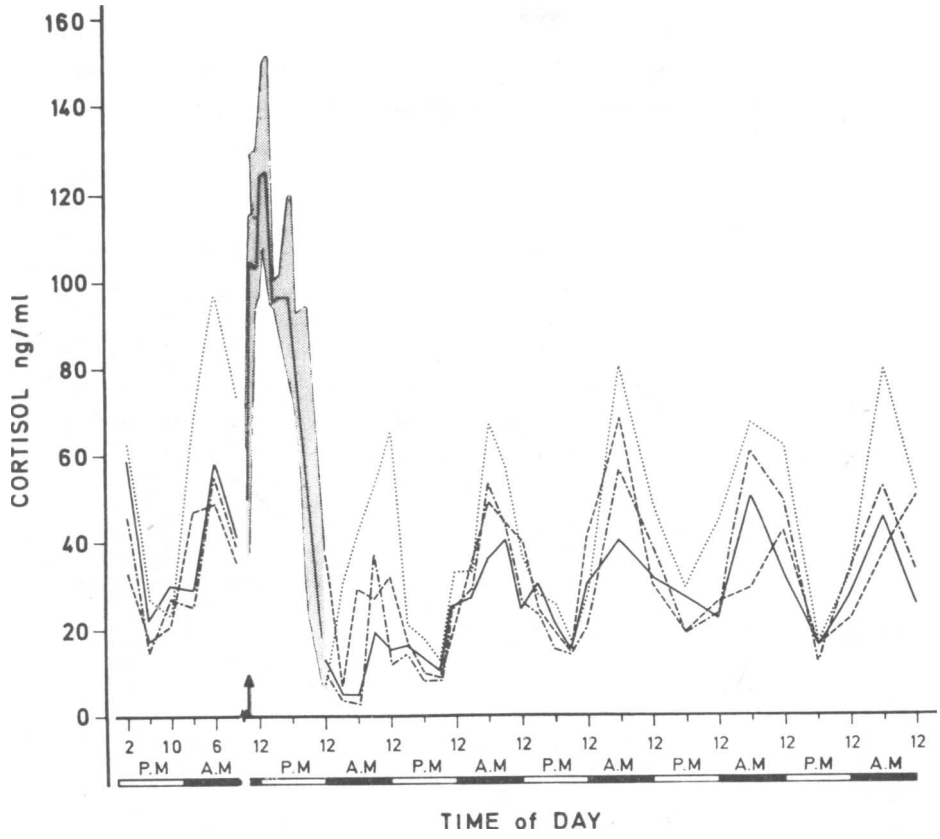
The plasma cortisol concentration and its diurnal variation is shown in Fig. 1 and Table 1. The cortisol content varied between 15 and 97 ng/ml with peak values at 6 a.m. (mean 65 ng/ml; range 49—97 ng/ml) and lowest values at about 6 p.m. (mean 20 ng/ml; range 15—27 ng/ml). The mean plasma cortisol concentration based on sampling at 4 hr. intervals during the 24 hr. period for the 4 horses was 42 ng/ml (s ± 20 ng/ml).

Table 1. Diurnal variation in plasma cortisol concentration in the 4 horses.

Time of the day	Cortisol concentrations (ng/ml)		
	mean	s	range
2 a.m.	42	19	25—67
6 a.m.	65	22	49—97
10 a.m.	47	18	35—74
2 p.m.	50	14	33—63
6 p.m.	20	5	15—27
10 p.m.	25	4	21—30
24 hrs.	42	20	15—97

Serial sampling in 1 horse during 30 min. resulted in a mean of 85 ng/ml with a standard deviation of 5.9 %.

The effects of the ACTH injection on cortisol levels are shown in Fig. 1 and Table 2. The peak concentrations occurred between 2 and 4 hrs. after injection. The mean of the maximum values was 140 ng/ml (range 131—151 ng/ml; Table 2). This mean value markedly exceeds both the current basal level and the mean plasma concentration during a 24 hr. period. After this peak the cortisol concentrations decreased to a minimum at 14—20 hrs. after injection (12 p.m.—6 a.m.; Fig. 1). The lowest mean concentration after stimulation was obtained at the 17th postinjection hour (11 ng/ml). The expected basal concentration



F i g u r e 1. Individual plasma cortisol levels before and after intramuscular injection of 150 i.u. of long-acting ACTH (arrow). Following injection of ACTH the mean (solid line) and range (shaded area) of cortisol levels in the 4 horses are given.

at the same time of the day according to the prestimulation study would be 47 ng/ml (Fig. 2). The next peak occurred at the 23rd hour after injection (mean 34 ng/ml). The corresponding mean concentration at this time (9 a.m.) would be 51 ng/ml. At the 35th postinjection hour (9 p.m.) a new minimum level was obtained with a mean cortisol concentration of 10 ng/ml as compared to the expected value of 24 ng/ml. Thus during the immediate 24 hrs. after the induced cortisol peak the levels of the hormone were markedly lower and out of phase with the basal diurnal pattern. Thereafter the cortisol pattern in the treated horses gradually adjusted to the pattern found in the prestimu-

lation study, and on day 3 after injection no differences between the patterns were detected.

After injection of 300 i.u. of ACTH in 1 horse the peak concentration of cortisol (163 ng/ml) was obtained at the 5th post-

Table 2. Plasma cortisol concentrations in horses before and after intramuscular injection of 150 i.u. of long-acting ACTH.

Horse No.	Cortisol concentration before injection of ACTH ng/ml	Maximum cortisol concentration after injection of ACTH		Minimum cortisol concentration after injection of ACTH	
		ng/ml	time after injection	ng/ml	time after injection
1	70	131	2 hrs.	8	14 hrs.
2	44	142	3 hrs.	7	17 hrs.
3	49	151	4 hrs.	3	20 hrs.
4	36	135	4 hrs.	4	17 hrs.
1*	83	163	5 hrs.	3	24 hrs.

* Intramuscular injection of 300 i.u. of the same ACTH preparation.

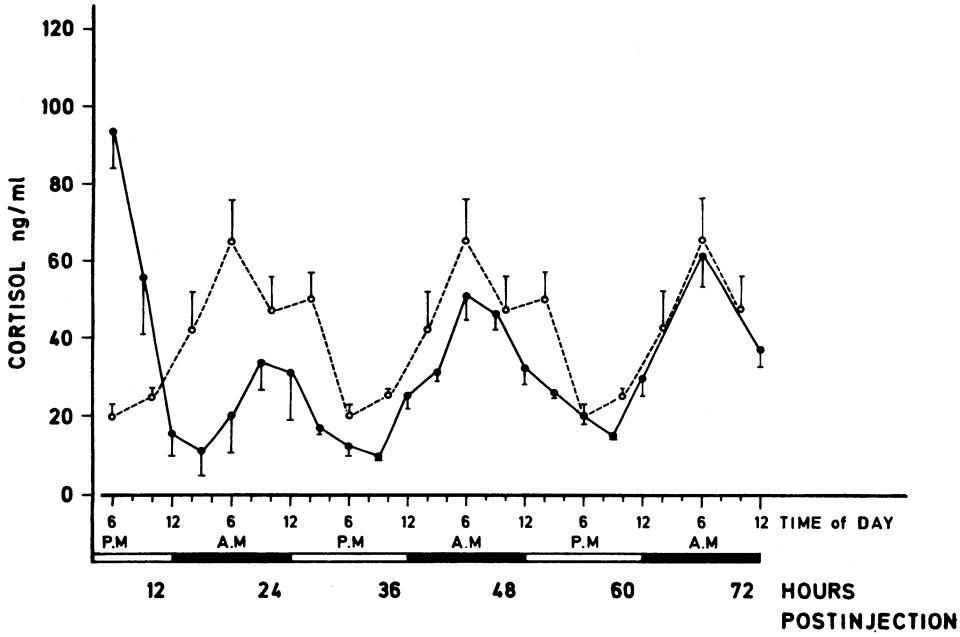


Figure 2. Mean plasma cortisol levels in 4 horses before and after intramuscular injection of 150 i.u. of long-acting ACTH. Solid line indicates mean cortisol level between 8 and 72 hrs. after injection of ACTH. Dashed line indicates mean cortisol level during the prestimulation study. Vertical bar indicates standard error of the mean value.

injection hour (Table 2). The period in which high cortisol values were found was somewhat prolonged as compared to when the same horse was given 150 i.u. of ACTH. Nine hrs. after injection the cortisol concentration was still 145 ng/ml. The lowest level of cortisol (3 ng/ml) was found at the 24th post-injection hour.

DISCUSSION

The mean plasma concentration of cortisol for normal horses obtained in the present study (42 ng/ml; s ± 20 ng/ml) compares favorably with the results in previous reports by authors using the competitive protein binding technique (*Hoffsis et al.* 1970), but are not in agreement with those found by authors using thin layer chromatography, ultraviolet absorption and fluorescence (*Zolovick et al.* 1966). *Hoffsis et al.* found a mean plasma cortisol concentration of 51.2 ng/ml (s ± 16.7 ng/ml) in normal horses and a well-defined diurnal rhythm with the highest values at 8 a.m. and lowest at 4 p.m. *Zolovick et al.* found values varying between 2190 and 3953 ng/ml with maximum at 10 a.m. and minimum at 8 a.m. The bleeding intervals of 28 hrs. used by *Hoffsis et al.* in order to eliminate the variation due to frequent sampling in measuring the diurnal rhythm of cortisol concentration, were not used here. In the present experiment the sampling with 4 hr. intervals did not seem to alter the results, as there was a consistent pattern of diurnal variation in all horses. This could partially be explained by the fact that we used experimental horses accustomed to the clinical routines and frequent blood sampling. Using adult horses of the same sex and breed might also have reduced some of the interindividual variations.

Judging from the fluctuations of cortisol levels during the day, a single measurement of plasma corticoids is of little clinical value. More complete information can be obtained by bleeding at specific intervals in order to measure the diurnal variation, or even better, measure the adrenocortical response to exogenous ACTH. In order to choose an adequate dose of ACTH in our experiment, a survey of the available litterature was performed, but little information could be found on dosage of ACTH in horses. *Hoffsis et al.* used 100 and 500 i.u. and *Snow & Munro* (1975) 100 i.u., both dosages yielding a marked corticoid response. *Paape et al.* (1977) found that 100 to 200 i.u. were re-

quired to produce a prolonged increase of the plasma corticoid level in the cow. In different studies in dogs (*Richkind & Edqvist* 1973, *Schwartz-Porsche et al.* 1976, *Sallman et al.* 1977) doses from 1.5 up to about 10 i.u. per kg body weight have been used. In the present study the dose of 150 i.u. was chosen, as the dosage commonly used in equine practice varies between 150 and 300 i.u. In this study a dose of 300 i.u. was given to 1 horse for comparison.

As seen in Fig. 1, a rapid response was obtained after administration of 150 i.u. of ACTH, and within 1 hr. the corticoid levels differed significantly from the basal values. The mean peak level (140 ng/ml) was reached 4 hrs. after the injection and the cortisol levels were still significantly higher than the basal levels about 8 hrs. after injection (Fig. 2). These findings seem to indicate that 150 i.u. of long-acting ACTH i.m. is sufficient to produce a therapeutically adequate response in the blood cortisol level. The single injection of 300 i.u. in 1 horse resulted in a more marked increase of the cortisol concentration and the peak was slightly prolonged as compared to the response to 150 i.u. of ACTH. However, after 24 hrs. the 2 dose levels showed an identical pattern of plasma cortisol concentrations.

The cortisol level reached the base line in about 12 hrs. Thereafter it continued to drop, and concentrations markedly below the expected level were found during the next 24 hrs. (Fig. 2). The rebound depression has not been described earlier in the horse but is known to occur in man, where on the second and third day after i.v. infusion of ACTH, a drop in the 17-ketosteroid excretion in urine, increased sodium excretion, potassium retention and high eosinophilic count have been described (*Renold et al.* 1952).

The material used here is too small to establish whether the rebound depression is to be regarded as a constant phenomenon or not. It seems likely, however, that it should occur in the horse as well as in man.

The diurnal rhythm of the cortisol concentration seems also to have been affected during the second and third postinjection day (Fig. 1). Before injection the peak concentration in the 4 horses occurred at 6 a.m., but after injection of 150 i.u. of long-acting ACTH at 10 a.m. the peak level after the injection was found between 9 and 12 a.m. On the third day, however, the rhythm was restored.

It seems that the stimulating effect of long-acting ACTH given by intramuscular injection at a dose of 150 i.u., lasts no longer than 12 hrs., when judged by the plasma cortisol concentration, only. On the other hand, the rebound effects persist for at least another 36 hrs. and should be regarded as a significant action of the drug. Since this effect persists less than 4 days, and since it is not possible to detect increased cortisol levels after 12 hrs., it seems unwarranted, in our opinion, to put long-acting ACTH in the "14-days-group" mentioned in the introduction.

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SAMMANFATTNING

Cortisol i hästplasma, dygnsrytm och inverkan av ACTH-injektion.

Cortisolkoncentrationen i perifer blodplasma och dess dygnsvariation mättes på fyra hästar. Medelkoncentrationen av cortisol under 24 timmar var 42 ng/ml (s ± 20 ng/ml). Högsta värdena uppmättes klockan 06 och lägsta omkring klockan 18 (medelvärden 65 ng/ml respektive 20 ng/ml).

Vid i.m. injektion av 150 IE långtidsverkande ACTH erhölls maximala cortisolkoncentrationer efter 2 till 4 timmar hos de fyra hästarna. Under de följande 24 timmarna var cortisolnivåerna markant lägre och dygnsrytmen förskjuten i förhållande till det basala mönstret. Under andra dagen efter injektionen skedde en gradvis justering och tre dagar efter injektionen kunde inga skillnader mellan de båda mönstren påvisas.

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