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PLASMA CONCENTRATIONS OF TESTOSTERONE IN THE MALE DOG AND PLASMA TESTOSTERONE PROFILE FOLLOWING SINGLE INTRAMUSCULAR INJECTION OF HCG*

By

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SUNDBY, A. and T. ULSTEIN: *Plasma concentrations of testosterone in the male dog and plasma testosterone profile following single intramuscular injection of HCG*. Acta vet. scand. 1981, 22, 409—416. — The spontaneous variation in plasma testosterone was studied in 4 dogs in a 24 h period. Blood samples were taken with 1½ h interval. A variation of 26—62 % was found in the plasma testosterone concentration and the values ranged from 2.7—15.6; 0.7—10.4; 4.2—17.3; 8.7—23 nmol/l. No effect of intramuscular injection of 150 i.u. HCG could be seen on the plasma testosterone levels in samples taken 5, 10, 20 or 30 min post injection. Thereafter plasma testosterone levels increased to reach levels equal to peak concentration in the control period 1½ h post injection. The variation in the plasma testosterone concentration 1½, 3 and 4½ h post HCG injection was reduced to 8.95 ± 2.8 % (mean \pm s). Plasma testosterone in a sample taken 3 h after HCG injection might thus be indicative of a Leydig cell dysfunction in the dog. An additional increase ($P < 0.001$) in plasma testosterone levels was observed 21—30 h post injection. Thereafter levels of testosterone fell to pre-stimulation values.

dog; plasma testosterone; HCG injection.

With a view to using measurement of plasma testosterone as a tool for clinical diagnosis of testicular dysfunction in dogs, it is necessary to study normal plasma testosterone level in this species. Moderate or extensive variation in plasma testosterone concentration during 24 h periods found in a number of species, including: man (Rowe *et al.* 1974), monkey (Wilson *et al.* 1978), rat, mice (Bartke *et al.* 1973), rabbit (Rowe *et al.* 1975, Moor &

* The investigation was financially supported by the Agricultural Research Council of Norway.

Younglai 1975), male lamb (*Foster et al.* 1978), ram (*Katongole et al.* 1972, *Purvis et al.* 1974), bull (*Katongole et al.* 1971, *Sanwal et al.* 1974, *Thibier* 1976) and male marsupials (*Lincoln* 1978), and while this work was in progress, also in dog (*DePalatis et al.* 1978). HCG stimulation was found to eliminate the extensive variation in plasma testosterone in the bull (*Sundby et al.* 1975). A purpose of the present investigation was to see whether a similar response occurs in the dog. Testosterone secretion in response to HCG infusion in the spermatic artery in the dog has been extensively studied (*Brinck-Johnsen & Eik-Nes* 1957, *Eik-Nes* 1967). However, the plasma testosterone profile following a single HCG injection and a possible period with steady plasma testosterone concentration has not been investigated previously.

MATERIALS AND METHODS

Eight adult male dogs were studied. The dogs were raised in a dog kennel (of Dr. N. Koppang), and brought to the Veterinary College a few days before the experiments. In the first experiment 4 dogs (3 Labrador and 1 Pointer) were sampled with 1½ h intervals for 24 h. In the second experiment 4 dogs (3 Labrador and 1 English setter) were sampled in a control period (7 samples with 1½ h intervals for each dog). One blood sample was then taken immediately prior to intramuscular injection of 150 i.u. HCG and sampling were continued at 5, 10, 20, 30, 40, 50 and 60 min, then with 1½ h intervals from 1½—9 h post injection, then with 3 h interval from 9—30 h post injection and additional sampling 48, 51 and 125 h after injection. All samples were taken from cephalic veins using vacutainers (Vacutainer, Becton Dickinson LTD., France). Following centrifugation at 1800×g in 10 min the supernatant plasma was stored at -20°C until analysing. Testosterone was assayed by the radio-immunoassay of *Sanwal et al.* (1974) as modified by *Sundby et al.* (1975).

Peripheral plasma HCG levels were measured at Aker Hospital, by Dr. P. Torjesen (*Sand & Torjesen* 1973).

RESULTS

The plasma testosterone profiles in 4 dogs throughout a 24 h period are shown in Fig. 1. Ranges, mean values, number of

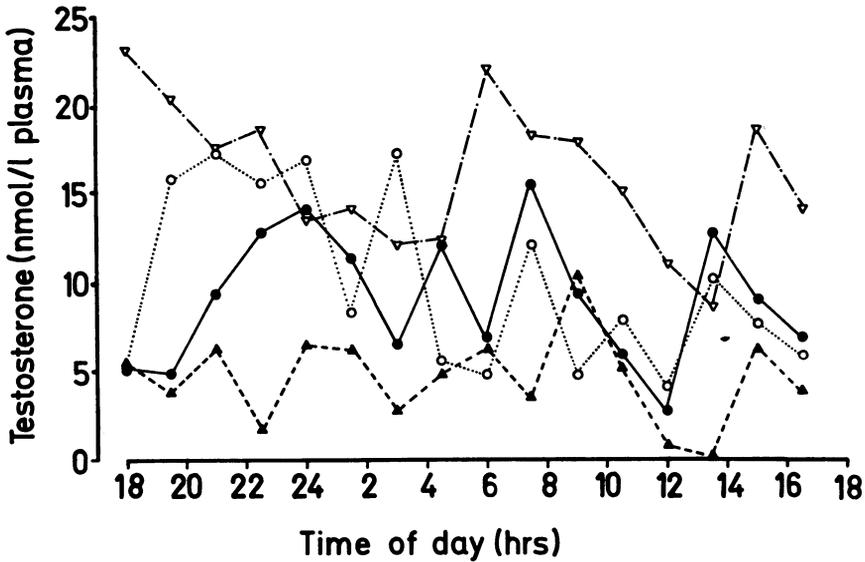


Figure 1. Plasma testosterone profiles in 4 dogs in a 24 h period.

peaks, peak elevation defined as per cent change from nadir to peak, and time from nadir to peak are listed in Table 1. A moderate fluctuation (26—62 % variation) in plasma testosterone was found. Three dogs had peak values in plasma testosterone around the hour 6—7, 13—14 and 22—23 of the day. The fourth dog (B) had generally low plasma testosterone concentrations with 5 peaklets during the 24 h period.

Fig. 2 shows the effect of intramuscular injection of 150 i.u. HCG plasma testosterone levels in 4 dogs. During the control

Table 1. Characteristics of plasma testosterone concentrations in 4 male dogs measured with 1½ h interval in a 24 h period.

Dog	Plasma testosterone			Number of peaks	Peak elevation % change from nadir to peak	Time from nadir to peak (min)
	nmol/l	variation $s \times 100$	%			
	Range	$\bar{x} \pm s$	\bar{x}			
A	2.7—15.6	9.4±3.8	41	4	64±14	135±90
B	0.7—10.4	4.5±2.8	62	5	65±19	90
C	4.2—17.3	10.4±5.2	50	4	55±13	113±45
D	8.7—23	16.3±4.2	26	3	50± 6	180

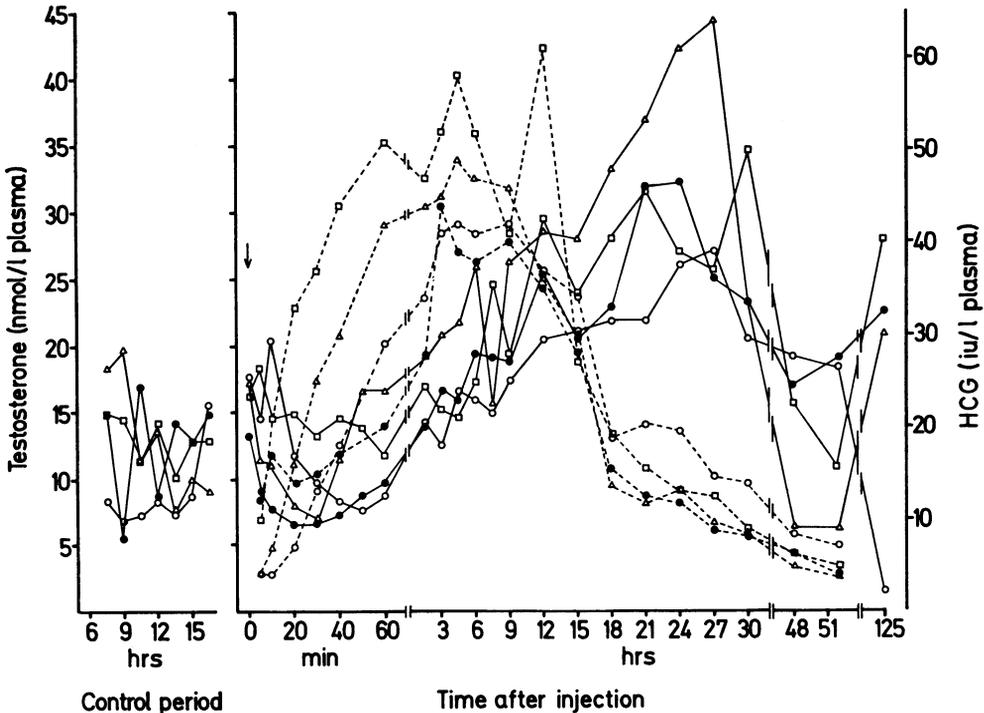


Figure 2. Plasma testosterone profiles in 4 dogs in a control period and following i.m. injection of 150 i.u. HCG (—). Plasma HCG profiles in the 4 dogs (----).

period plasma testosterone fluctuated slightly (14–35 % variance, $n=7$). No effect of HCG upon plasma testosterone levels could be seen in samples taken 5, 10, 20 or 30 min after injection. In 3 dogs increasing plasma testosterone concentration was observed from 40–60 min post injection, while in the fourth dog plasma testosterone concentration was kept on a high, slightly fluctuating level. Levels equal to peak concentration in the control period were reached 1½ h post injection. In these 4 dogs the variation in plasma testosterone levels 1½, 3 and 4½ h post HCG injection was 8.95 ± 2.8 % (mean \pm s) compared to a variation of 31 ± 23 % in the control period at corresponding points in time during the day. A further rise to peak value of 3 times maximal control plasma testosterone concentration was observed 21–30 h post injection. Thereafter levels of testosterone fell to prestimulation values. The testicular responsiveness to intramuscular injection of 150 i.u. HCG in dogs is thus seen as

a first increase ($P < 0.001$) 1½—6 h post injection, and an additional increase ($P < 0.001$) 21—30 h post injection.

Plasma HCG concentration increased after a single intramuscular injection of 150 i.u. HCG to a level of 45—55 i.u. HCG/l 1—3 h post injection, remained on this level until 10—14 h post injection. Thereafter plasma HCG concentration fell, and a level of around 10 i.u. HCG/l plasma was measured at the time of maximal plasma testosterone concentration (21—30 h post injection). In the period 15—18 h post injection the fall in HCG concentration corresponded to a half-life of HCG of about 3½ h.

DISCUSSION

Plasma testosterone was found to fluctuate with 3—5 peaks (change in elevation from nadir to maximum of 39—88 %) during a 24 h period. The plasma testosterone range was in agreement with that reported by *DePalatis et al.* (1978). However, in the latter investigation 3 dogs were sampled every 20 min and by defining peaks as an elevation from nadir to maximum of at least 25 %, 10 to 18 peaks were found in the 24 h period studied. The variation in plasma testosterone during the day is not sufficiently consistent to make measurement of plasma testosterone in single samples at any fixed time suitable for an evaluation of testicular function. In the bull a single HCG injection was found to reduce the variation in plasma testosterone for a 12 h period (*Sundby et al.* 1975) and single sampling at a fixed time following HCG injection was found to be suitable for comparing plasma testosterone between bulls (*Sundby & Velle* 1980). In dogs the variation in plasma testosterone following intramuscular administration of 150 i.u. HCG was reduced during a short period only (1½—4½ h post injection). However, plasma testosterone in a sample taken within this period, for instance 3 h post injection, would be indicative of a testicular malfunction. Whether this level is significantly related to control plasma testosterone concentration, as shown for the bull (*Sundby & Velle* 1980) remains to be investigated. No effect of HCG upon plasma testosterone levels in dogs was observed in samples taken 5, 10, 20 or 30 min post injection. This is similar to the observation in bulls (*Sundby* 1981). However, *Eik-Nes* (1967) and *Boyden et al.* (1980) found increased testosterone secretion in dogs within 10 min following intravenous infusion

of HCG into spermatic artery and following a single injection of 25 i.u. HCG into the spermatic vein. A plateau level equal to the peak concentration in the control period reached 1½ h after intramuscular injection is similar to that observed in bulls (Sundby 1981). However, a secondary effect of HCG seen as a further rise in plasma testosterone appeared much earlier post HCG injection in the dog than in the bull, 21—30 h versus 3—5 days, respectively (Sundby & Farahat 1978). The plasma testosterone profile following HCG injection in dogs were on the other hand quite similar to the testicular response to HCG seen in the plasma 5 α -androstenedione and testosterone profiles in pigs (Andresen 1975). This might be due to species differences in the half-life of HCG, and in the rate of HCG induced enzyme synthesis. Peak levels of plasma testosterone occurring at a time when HCG concentration has fallen to very low levels is similar to that reported in the bull (Sundby & Torjesen 1978).

ACKNOWLEDGEMENTS

Thanks are due to Dr. N. Koppang for providing the dogs and to K. Nordqvist for help during sampling. The technical assistance of Randi Løes Skogstad is greatly appreciated.

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SAMMENDRAG

Plasma testosteron konsentrasjoner hos hund og plasma testosteron profil etter en enkel intramuskulær HCG injeksjon.

Variasjonen i plasma testosteron ble studert hos 4 voksne hannhunder i en 24 timers periode. Blodprøver ble tatt med 1½ times mellomrom. Plasma testosteron konsentrasjonen svingte mellom 2,7—16,6; 0,7—10,4; 4,2—17,3; 8,7—23 nmol/l. Verdiene viste en varians mellom 26—62 %. Ingen effekt av intramuskulær injeksjon av 150 i.u. HCG kunne påvises i blodprøver tatt 5, 10, 20 eller 30 min etter injeksjonen. Deretter steg nivået av testosteron i plasma og nådde 1½ time etter HCG injeksjonen et nivå likt toppkonsentrasjonen i kontrollperioden. Variansen i plasma testosteron konsentrasjonen 1½, 3 og 4½ time etter time etter HCG injeksjonen var redusert til $8,95 \pm 2,8$ % (gjennomsnitt \pm s). Plasma testosteron i en prøve 3 timer etter HCG injeksjon vil kunne indikere en dysfunksjon i Leydigcellene hos hund. En ytterligere øking ($P < 0,001$) i plasma testosteron nivå ble observert 21—30 timer etter injeksjonen. Deretter falt testosteron nivået til verdier målt i kontrollperioden.

(Received June 22, 1981).

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