Effects of Hemicastration or hCG-treatment on Steroids in Testicular Vein and Jugular Vein Blood of Stallions

RUPERT P. AMANN AND VENKATASESHU K. GANJAM*

The relationships among testicular vein and jugular vein concentrations of androgens and estrogens were studied in anesthetized stallions. Blood was sampled from a vein on the surface of the testis and simultaneously from an artery on the surface of the testis and from the jugular vein. Concentrations of total 17β-hydroxy-androgens and total estrogens were measured for all samples and testosterone, dihydrotestosterone, 3α-andostanediol, 3β-androstanediol, and 5-androstenediol were quantified in selected samples. Following halothane anesthesia and hemicastration of 19 stallions, the testosterone concentration in jugular blood dropped over 3 hours but returned to normal within two days. Although jugular levels of total 17β-hydroxy-androgens were normal (~1.4 ng/ml) 12 days after hemicastration, the concentration of total 17β-hydroxy-androgens in testicular vein blood was sixfold greater (P < 0.01) than it had been at hemicastration (368 vs 62 ng/ml). The ratio of testosterone to 5α-reduced-androgens was similar in testicular vein blood from the first and second testes. Apparently, the clearance rate for testosterone had changed from ~2.4 l/min/stallion to ~6.2 l/min/stallion in 12 days. Concentrations of total estrogens in testicular vein blood were similar for the first and second testes (27,400 pg/ml) as compared with the values for jugular vein and testicular artery blood (63 and 293 pg/ml, respectively). Clearance rate of estrogens (~23.3 l/min in intact stallions) ap-

From the Animal Reproduction Laboratory, Department of Physiology and Biophysics, Colorado State University, Fort Collins, Colorado; and the Section of Clinical Reproduction, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, Pennsylvania

parently decreased by 50%. Although anesthesia plus hemicastration may have altered blood flow to the remaining testis, a change in blood flow could not account for the concurrent increase in testosterone and decrease in estrogen clearance rates. Injection of 1500 IU of hCG markedly increased (P < 0.01) the concentration of total 17β-hydroxy-androgens in testicular vein blood 1 hour later, but the concentration of total estrogens was unaltered 1 or 2 hours after treatment. We concluded that the concentrations of total 17β-hydroxy-androgens and total estrogens in jugular blood bear little relationship to their concentrations a few minutes earlier in venous blood draining the testis.

Recent studies have documented the occurrence of episodic elevations of testosterone in peripheral blood from stallions (Squires et al, 1977; Ganjam, 1979), but there is conflicting evidence (Kirkpatrick et al, 1976; Sharma, 1976; Enbergs et al, 1977; Squires et al, 1977) concerning the presence of diurnal variation in the basal concentration of testosterone in stallion blood. There is no information for stallions on the increase in testicular steroidogenesis necessary to produce a detectable change in levels of testicular hormones in peripheral blood or on the correlation between

steroid concentrations in testicular venous blood and in jugular blood. Steroidogenesis in the equine testis is of interest because of the uniquely large amounts of estrogens produced (Nyman et al, 1959; Bedrak and Samuels, 1969; Oh and Tamaoki, 1970). Since equine blood may not contain a specific globulin with a high affinity for testosterone or estradiol (Corvol and Bardin, 1973), the relationship between testicular venous and peripheral blood levels of steroids might be different than in a species with a specific steroid-binding globulin in blood.

Based on studies with other species (Setchell, 1978), we assumed that blood levels of LH would rise following hemicastration and that testicular production of steroids might be increased in response to this stimulus. Injection of hCG into intact stallions was used as an alternative method to stimulate steroidogenesis. Within 10—90 minutes after injection of hCG into rats or bulls, there are twofold increases in the concentrations of both testosterone and estradiol in testicular venous blood (deJong et al, 1973; Amann and Ganjam, 1976). The principal objectives of this research were to investigate a) the effects of hemicastration and hCG administration on the steroid profile of testicular venous blood and b) the relationships among concentrations of steroids in testicular vein and jugular vein blood. Preliminary reports of these data have been presented (Amann and Ganjam, 1977; Ganjam and Amann, 1979).

Materials and Methods

Clinically normal two- to 16-year-old stallions, of light horse breeds, were used for two experiments conducted between late May and early September in 1975 and 1976. Repeated sampling of testicular vein blood through an indwelling catheter was rejected for technical reasons. Although limited to a single time point, direct sampling from the subcapsular portion of a testicular vein provided blood undiluted by blood draining the epididymis.

Experiment 1

The effects of hemicastration on blood levels of steroids were evaluated using 20 stallions (Amann et al, 1977). A 12-day interval was allowed before removal of the second testis. One stallion died following hemicastration; data for this animal were excluded.

Samples of jugular blood were taken by venipuncture 1 hour before hemicastration, again when the stallion was placed in dorsal recumbancy, and again when the first testis was removed. Additional blood samples were taken every 30 minutes for the next 4 hours, hourly

through 12 hours, after 24 to 30 hours and in midafternoon each day thereafter.

For castration, a stallion was given sodium thiamylal (Surital, Parke-Davis), and anesthesia was maintained with halothane (Fluothane, Ayerst). The stallion was placed in dorsal recumbancy and prepared for surgery. The testis removed at hemicastration was selected on an alternating basis. Using aseptic technique, blood vessels lying within the tunica albuginea of the selected testis were exposed by incising the scrotum and tunica vaginalis parietalis (Amann and Ganjam, 1976). Samples (10—12 ml) of blood were withdrawn over 2—3 minutes through a 22-gauge needle attached to a 12-ml syringe. Blood from a major vein on the testicular surface was taken before blood from the superficial portion of the testicular artery; jugular blood was taken during withdrawal of blood from the testicular artery. The testicular artery and vein then were ligated above the pampiniform plexus, and the testis was extripated. Tissue was brought into apposition to close the void space, and successive layers were meticulously sutured. Little or no postsurgical swelling was noted. Blood samples were taken similarly from the second testis 12 days after hemicastration. Blood was allowed to clot at room temperature and held at 5 C before recovery of serum.

Experiment 2

The effects of hCG on blood levels of steroids were studied using 24 stallions (Squires et al, 1978). Stallions were randomly assigned to one of three hCG-treatments: 1500 IU 1 hour before the anticipated time of castration, 1500 IU 2 hours before castration, or a control injection of 0.14 M NaCl (saline) 2 hours before castration. Injection of hCG in saline or plain saline was intravenous. Samples for one stallion injected with saline were lost.

Samples of jugular blood were taken at −4, −3, −2 and −1 hours before the planned time of castration. A stallion was given approximately 400 mg of xylazine (Rompun, Haver-Lockhart) intramuscularly, followed by intravenous administration of 700—1000 ml of a mixture of sodium thiamylal (3 g/l, Surital; Parke-Davis) and glyceryl guaiacolate (Guaifenesin, Gan's Chemical Works). Bilateral castration and blood sampling proceeded as described under Experiment 1, except that the stallion was in lateral recumbancy, asepsis was not maintained, and samples of testicular vein, testicular artery, and jugular vein blood were taken as each testis was removed. Blood was taken from the testicular vein of the first testis within 8 ± 1 minutes of the planned time for castration and from the second testis 10 ± 1 minutes later. Blood was immediately cooled in ice, and serum was harvested 15 to 18 hours later.

Hormone Analyses

Sera from jugular blood taken before hemicastration and between removal of the first and second testes from stallions in Experiment 1 were radioimmunoassayed for testosterone (Thompson et al, 1977). These samples

were not chromatographed since the avidity of the testosterone antibody for dihydrotestosterone was 25% that of testosterone (cross-reactivity of other steroids was <26%), and the dihydrotestosterone concentration in stallion jugular blood was found to be about 14% that of testosterone; 3α- and 3β-androstanediol were present in even lower concentrations (unpublished data).

For both experiments, sera from samples representing each testis (testicular vein, testicular artery, and jugular vein) were evaluated for total 17β-hydroxy-androgens by a competitive protein-binding assay (Ganjam, 1976) and for total estrogens by radioimmunoassay (Ganjam and Kenney, 1975). The rabbit testosterone-binding globulin used for quantification of total 17β-hydroxy-androgens bound $3\beta,17\beta$-dihydroxy-androst-5-ene (5-androstenediol), dihydrotestosterone, 3α-17β-dihydroxy-5α-androstane (3α-androstanediol), and $3\beta,17\beta$-dihydroxy-5α-androstane (3β-androstanediol) with a avidity of 60, 300, 120, and 75% that of testosterone, respectively (Ganjam, 1976). Although total 17β-hydroxy-androgens were expressed as testosterone equivalents, similar steroids with a 17β-ol structure could contribute to the values presented. For selected samples, individual androgens were isolated by Sephadex LH-20 chromatography (Ganjam, 1976), and concentrations of testosterone, dihydrotestosterone, 3α-androstanediol, 3β-androstanediol, and 5-androstenediol were measured (Ganjam and Kenney, 1975; Ganjam, 1976) by competitive protein-binding assays.

The antiserum used to assay total estrogens had an avidity for estrone, estradiol, estriol, equilin, and equilenin of 94, 100, 18, 27, and 27%, respectively (Ganjam and Amann, 1976). Thus, total estrogens represent primarily estradiol and estrone and were expressed as estradiol equivalents.

All assays were validated for use with stallion plasma. Serial dilutions of plasma extracts and additions of known amounts of standard steroids to plasma samples both resulted in curves parallel to those for standard in buffer. All intra- and interassay coefficients of variations were $\leq 12\%$. Analyses for testosterone (isolated by high performance liquid chromatography (HPLC)) and for total androgens in plasma extracted within 30 minutes after bleeding or extracted after storage at 5 C for 24 hours revealed no significant difference over time (898, 1026, 880, and 1008 ng/ml; n = 6 samples).

Statistical Analyses

A one-way analysis of variance was used to evaluate effects of age (2–3, 4–9, and 11–14 years old) on the hormonal content of blood from the three sites. For Experiment 2, a one-way analysis of variance considered hCG-treatment (none, 1, or 2 hours before castration). For each characteristic, differences between the first and second testis were evaluated using a t test for paired data. Correlation coefficients also were calculated.

Results

Effects of Hemicastration

After anesthesia and hemicastration, the concentration of testosterone in jugular blood dropped over the next 3 hours. Testosterone concentration remained low until about 9 hours after hemi-
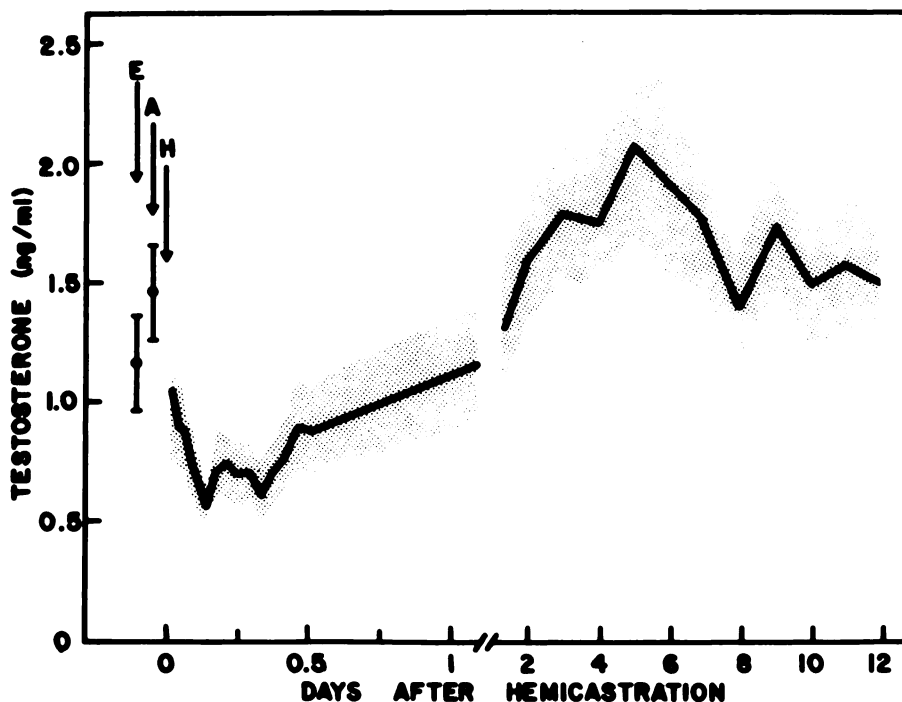

Fig. 1. Effects of anesthesia and hemicastration on testosterone concentration in jugular blood. Mean ± SEM for 19 stallions. The first three data points are for blood taken about 1 hour before castration (E), just after induction of anesthesia (A), and at the time of hemicastration (H). Testosterone concentration in four samples of jugular blood taken one day to two months earlier from each of the 19 stallions averaged 2.30 ± 0.33 ng/ml.

castration and then rose (Fig. 1). Values approached normal level by 24–30 hours, and by two days after hemicastration the concentration of testosterone in jugular blood was similar (1.58 ± 0.25 ng/ml) to that for samples taken during the previous two months from the same stallions. Testosterone concentration in peripheral blood remained normal until removal of the second testis on day 12.

Testosterone concentration in jugular blood was similar ($P > 0.05$) at the time of hemicastration and 12 days later (Fig. 1). Similarly, concentrations of total 17β-hydroxy-androgens in jugular blood were the same when the second testis was taken as when the first testis was removed (Table 1). However, venous blood from the second testis contained more ($P < 0.01$) total 17β-hydroxy-androgens than that from the first testis (Table 1). Values for venous blood from 17 of 19 first testes were <120 ng/ml, while total 17β-hydroxy-androgens for 14 of 19 second testes were >160 ng/ml. Total 17β-hydroxy-androgens in blood from the testicular artery also were elevated. Thus, 12 days following hemicastration, the concentration of total 17β-hydroxy-androgens in venous blood from the remaining testis was sixfold greater than in venous blood from the first testis. Despite the remarkable elevation of total 17β-hydroxy-androgens in testicular venous blood, levels in jugular blood remained normal.

The effect of hemicastration on estrogen production by the testis was very different from that on androgen production. Concentrations of total estrogens were similar for both testes (Table 1) and averaged 63 ± 8, 293 ± 23, and 27,400 ± 3900 pg/ml for blood from the jugular vein, testicular artery, and testicular vein, respectively. Total estrogens in testicular venous blood were influenced by age of stallion ($P < 0.05$), and for the first testis

averaged 20,700, 36,000, and 17,700 pg/ml for stallions aged 2–3, 4–9, or 11–14 years.

For both first and second testes, concentrations of total 17β-hydroxy-androgens and total estrogens in testicular arterial blood exceeded ($P < 0.01$) those in jugular vein blood.

In general, correlation coefficients between concentrations of total 17β-hydroxy-androgens or total estrogens in blood from the three sampling sites were low (Table 2). Concentration of steroids in testicular venous blood accounted for less than 16% of the variability in steroid content of jugular vein blood. This fact was reflected in the wide ratio between the hormone concentrations in testicular venous and jugular blood taken at removal of the second testis. For total 17β-hydroxy-androgens, this ratio ranged from 3 to 760 and for total estrogens, from 114 to 1172. The highest correlation coefficient was for the relationship between total 17β-hydroxy-androgens in venous blood leaving the second testis and blood removed from the testicular artery below the pampiniform plexus ($r = 0.65$).

It was clearly demonstrated (Table 1) that the concentration of total 17β-hydroxy-androgens in testicular venous blood was markedly elevated following hemicastration. To determine whether this difference resulted from increased secretion of a specific steroid or a general elevation in concentrations of all hormones contributing to the total 17β-hydroxy-androgen pool, androgen profiles were determined for blood from two stallions. It was evident (Fig. 2) that the relative concentrations of identified steroids were similar for testicular venous and jugular blood and that the sevenfold increase in steroid concentration in venous blood from the second testis represented a general elevation of all steroids contributing to the total 17β-hydroxy-androgen pool. Thus, hemicas-

TABLE 1. Total 17β-hydroxy-androgens and Total Estrogens in Blood at Hemicastration and at Removal of the Second Testis 12 Days Later*

Source of Blood	Total 17β-hydroxy-androgens (ng/ml)		Total Estrogens (pg/ml)	
	First Testis	Second Testis	First Testis	Second Testis
Jugular vein	1.32 ± 0.09†	1.52 ± 0.19†	58 ± 12†	64 ± 9†
Testicular artery	1.79 ± 0.12	2.91 ± 0.35‡	294 ± 36	290 ± 30
Testicular vein	61.5 ± 13.6	368 ± 60.3‡	25,400 ± 3300	29,700 ± 7300

* Mean (± SEM) values for 19 stallions expressed as testosterone or estradiol equivalents. Variance associated with age of stallion was nonsignificant ($P > 0.05$) for each sampling site except for total estrogens in the testicular vein.
† Mean was lower than that for testicular artery or testicular vein ($P < 0.01$; t test for paired data).
‡ Mean for the second testis exceeded that for the first testis ($P < 0.01$; t test for paired data).

TABLE 2. Correlation Coefficients

	Total 17β-hydroxy-androgens			Total Estrogens		
	First Testis	Second Testis	Both Testes	First Testis	Second Testis	Both Testes
Jugular vein + testicular artery	0.29	0.59†	0.55†	0.47*	0.41	0.44†
Jugular vein + testicular vein	0.32	0.39	0.38*	0.24	0.34	0.27
Testicular artery + testicular vein	−0.03	0.65†	0.70†	0.37	0.02	0.12
Testicular weight + testicular vein	−0.16	0.22		0.32	0.34	0.31

* Correlation coefficient significant ($P < 0.05$).
† Correlation coefficient highly significant ($P < 0.01$).

tration did not alter the ratio of testosterone to 5 α-reduced-androgens. Although not representing a major component of the total 17β-hydroxy-androgen pool, the concentration of 5-androstenediol in venous blood from the second testis was more than twice that in venous blood from the first testis.

The most obvious difference in steroid profiles between jugular and testicular venous blood was in the proportion of unidentified steroids contributing to the total 17β-hydroxy-androgen pool. This value represented both a steroid with a 17β-ol structure, which eluted prior to dihydrotestosterone in our chromatographic system, and also the error inherent in the total 17β-hydroxy-androgen assay because 5α-reduced-androgens are bound with greater affinity than testosterone.

Effects of hCG

As anticipated, injection of hCG 1 or 2 hours before castration was followed by an elevation in the total 17β-hydroxy-androgen content of jugular blood (Fig. 3). At 1 hour after injection of hCG, total 17β-hydroxy-androgens in jugular blood had increased by 208 ± 30% (n = 16). However, concentrations of total estrogens were similar throughout the 5-hour sampling period, regardless of hCG-treatment. Injection of hCG before castration increased ($P < 0.01$) testosterone production by the testis, and this effect was more pronounced in testicular venous blood at 1 hour than at 2 hours after hCG injection (Fig. 4). Although the concentration of total 17β-hydroxy-androgens in testicular vein blood was higher ($P <$
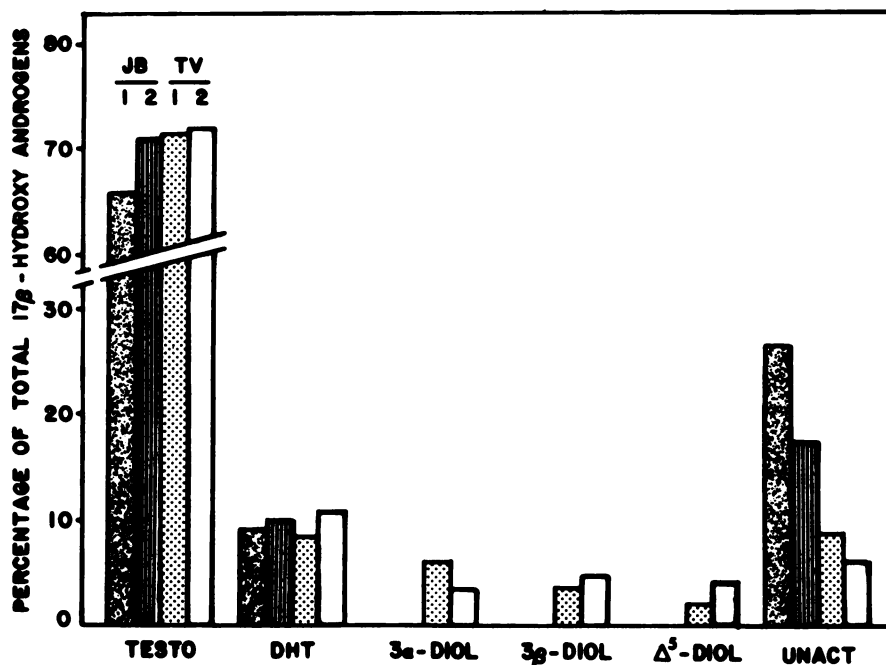

Fig. 2. Profile of androgens in stallion blood. Percentage contribution of the mass of individual steroids, assayed after chromatographic isolation, to the total 17β-hydroxy-androgen pool; unidentified steroid was obtained by difference and represents 17β-hydroxy-steroids bound by rabbit testosterone–estradiol binding globulin. Data for a second stallion were similar to those shown.
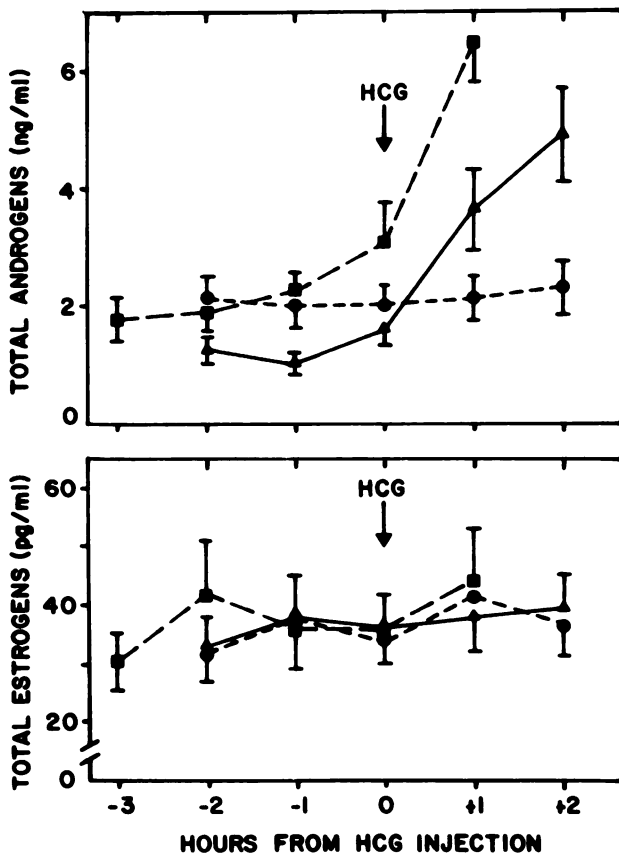
Fig. 3. Effect of hCG on blood levels of total 17β-hydroxy-androgens (upper) and total estrogens (lower). An injection of saline (●) or of hCG was given 2 hours (▲) or 1 hour (■) before castration. Mean ± SEM for seven or eight stallions per group.
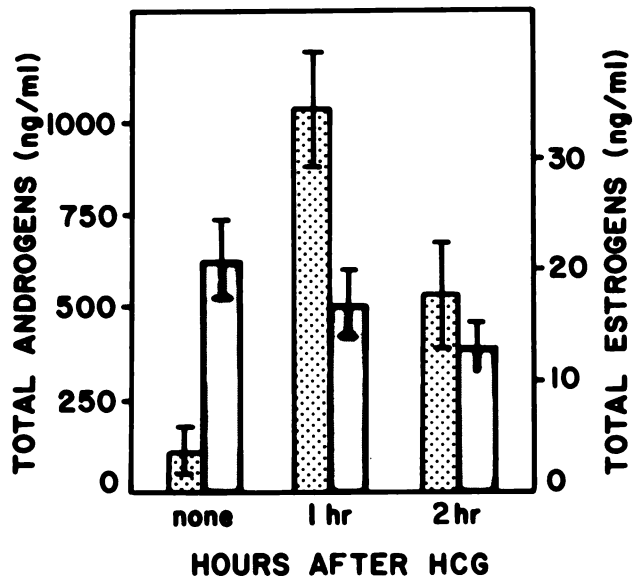

Fig. 4. Differential response of testicular steroidogenesis to hCG treatment. Mean ± SEM concentrations of total 17β-hydroxy-androgens or total estrogens in testicular venous blood from the first testis for seven or eight stallions per group.

0.01) for stallions castrated at 1 hour than for those castrated at 2 hours, the concentration in jugular blood was similar ($P > 0.05$) 1 and 2 hours after hormone injection for stallions injected 2 hours before castration. In contrast to the dramatic increase of total 17β-hydroxy-androgens (primarily testosterone; Fig. 2) in testicular venous blood, concentrations of total estrogens tended to decline ($P > 0.05$) (Fig. 4). Obviously, the concentration of estrogens in testicular venous blood was independent of testosterone production as represented by venous concentrations of total androgens ($r = 0.03$).

Correlations between concentrations of total 17β-hydroxy-androgens in blood from the jugular vein vs. the testicular artery, the jugular vein vs. the testicular vein, and the testicular artery vs. the testicular vein were 0.61, 0.72, and 0.86, respectively, for the first testis removed. Similar correla-

tions for total estrogens were 0.52, 0.26, and 0.58, respectively.

Discussion

From data presented, we concluded that concentrations of total 17β-hydroxy-androgens (primarily testosterone) and total estrogens in blood sampled from the jugular vein bear little relationship to their concentrations in blood draining the testis a few minutes earlier. The overall correlation (n = 84) between total 17β-hydroxy-androgen concentrations in testicular venous and jugular blood was 0.71, and that for total estrogens was only 0.32. Although part of this discrepancy surely represents attenuation of pulsitile discharge through the testicular vein by dilution with systemic blood, other factors must be involved.

The increased production of total 17β-hydroxy-androgens after hemicastration (Experiment 1) apparently was followed by an increased rate of steroid clearance; peripheral androgen levels were maintained at normal values. Clearance rate of total estrogens, however, probably declined by 50% since peripheral blood concentrations of total estrogens remained normal, while estrogen production by the remaining testis also was unaltered. An alternative explanation for the high concentration of total 17β-hydroxy-androgens in testicular venous blood 12 days after hemicastration

is that blood flow to the remaining testis decreased by 50%, and the clearance rate for androgens was unchanged. A 50% decrease in blood flow seems unlikely since the concentration of total estrogens was similar in venous blood draining the second and first testes. Although increased androgen production by the testis is not necessarily accompanied by increased estrogen production (Fig. 4), a 50% decrease in blood flow would have been accompanied by a 50% reduction in estrogen production to account for the concentration of total estrogens detected in testicular venous blood draining the second testis. Since the present experiments did not include sequential sampling of testicular vein blood or measurements of steroid clearance rates, the exact pattern of events in stallions remains unknown.

If blood flow to the testes of our stallions averaged 26 ml/min, as reported by Lindner (1961), the typical stallion in Experiment 1 secreted about 0.71 μg of estrogens/min/testis, while the production of testosterone (74% of the total androgens) was about 2.3 μg/min/testis when the first testis was removed. The estimated testosterone production rate for the first testes is consistent with the single observation of 2.2 μg/ml reported by Lindner (1961). By 12 days after hemicastration, the estimated rate of testosterone production was 7.0 μg/min/testis. Thus, the apparent testosterone clearance rate* increased from ~2.4 l/min/stallion to ~6.2 l/min/stallion in 12 days.

We anticipated that the testis remaining after hemicastration would double its production of steroids to compensate for loss of the first testis. Indeed, the concentrations of testosterone and

total 17β-hydroxy-androgens in jugular blood returned to normal by two days after hemicastration. However, as presented above, the testicular vein concentration of total 17β-hydroxy-androgens averaged sixfold higher 12 days after hemicastration, and in seven of 15 stallions was 15-fold greater. It is not known why androgen production by the remaining testis was stimulated, if this phenomena would disappear after a longer interval, or if similar stimulation occurs after hemicastrating males of other species. However, it is reasonable to assume that hemicastration and anesthesia were followed by induction of enzymes involved in hepatic clearance of androgens, while activity of enzymes involved in estrogen clearance was reduced.

Steroidogenesis in stallions obviously is very different from that in other mammals. Testicular venous blood from stallions contained about 21,200 pg/ml (n = 84) of total estrogens as compared with 10−100 pg estradiol/ml for bulls (Amann and Ganjam, 1976), rats (deJong et al, 1973), dogs and monkeys (Kelch et al, 1972), or approximately 1,000 pg/ml for humans (Kelch et al, 1972). Nevertheless, concentrations in jugular blood averaged only 49 pg/ml (n = 84) for stallions and 11 pg/ml for bulls (Amann and Ganjam, 1976). Presumably, estrogens are cleared very rapidly from stallion blood, and the estimated clearance rate of ~23.3 l/min is much greater than that of ~2.4 l/min estimated for testosterone in intact stallions.

The increase in the total 17β-hydroxy-androgen content of testicular venous blood induced by hCG treatment was greater than that following hemicastration. It is likely that injection of 1500 IU of hCG resulted in maximum stimulation of the Leydig cells, and jugular blood concentrations of testosterone attained 1 or 2 hours later (Fig. 3) were similar to those encountered during naturally occurring episodic releases (unpublished data). Consequently, the concentration of testosterone or total 17β-hydroxy-androgens in testicular venous blood during a naturally occurring burst of testosterone secretion probably approaches the 1000 ng/ml we found after hCG treatment (Fig. 4). This conclusion is consistent with observations for bulls (Amann and Ganjam, 1976).

Our experiments were not designed to evaluate the effects of anesthesia or dorsal vs. lateral recumbancy on steroid production. However, total 17β-hydroxy-androgen concentration in testicular

---

* Clearance rates of steroids from blood plasma were estimated by assuming that blood flow to the testis was a uniform 26 ml/min/testis (Lindner, 1961), that steroid production (PR) was represented by the difference between testicular vein and testicular artery concentrations times blood flow, and that clearance rate (CR) was the production rate divided by the concentration of steroid in peripheral blood. This calculation is independent of total blood volume. These calculations are valid only if the relative concentrations of steroids contributing to the total 17β-hydroxy-androgen and total estrogen pools were similar in testicular vein and jugular vein blood. For total 17β-hydroxy androgens or testosterone, the profiles were similar (Fig. 2) and, hence, the calculated values are reasonably accurate. It was assumed that testosterone represented 74% of the total 17β-hydroxy-androgens. For total estrogens, profile data are unavailable, and the reported values are accurate only if the profiles for total estrogens were similar in testicular vein and jugular vein blood. Using data from Table 1, for testosterone on day 0, clearance rate was calculated as: PR = [(61.5 ng/ml) − (1.8 ng/ml)] [0.74] [26 ml/min] [2] = 2297 ng testosterone/min/2 testes; CR = (2297 ng/min)/(1.3 ng/ml) (0.74) = 2351 ml/min.

venous blood from the first testis removed averaged 62 ± 14 ng/ml in Experiment 1 and 118 ± 31 ng/ml for stallions not receiving hCG in Experiment 2 ($P > 0.05$). Therefore, data for the first testis removed from stallions in Experiment 1 and from control stallions in Experiment 2 were combined to enable consideration of age-associated differences. For both total 17β-hydroxy-androgens and total estrogens, differences among means for stallions 2–3, 4–7, and 9–15 years old were not significant ($P > 0.05$), and correlations with age were low ($r = 0.14$ and 0.11).

In both experiments, the concentrations of total 17β-hydroxy-androgens and total estrogens in testicular artery blood exceeded ($P < 0.01$) those in jugular vein blood. Similar countercurrent transfer of steroids across the pampiniform plexus has been reported for other species (Amann and Ganjam, 1976; Free, 1977). For stallions, we calculated ($n = 84$) a transfer coefficient of $0.7 \pm 0.1\%$ for total 17β-hydroxy-androgens and $1.2 \pm 0.9\%$ for total estrogens. These values are lower than those for other species. Considering the low transfer coefficients, the point where epididymal arteries leave the pampiniform plexus (Ippensen et al, 1972), and data for other species (Free, 1977), the countercurrent transfer of steroids across the pampiniform plexus probably has no physiologic significance in stallions.

Acknowledgments

References

Amann RP, Ganjam VK. Steroid production by the bovine testis and steroid transfer across the pampiniform plexus. Biol Reprod 1976; 15:695–703.

Amann RP, Ganjam VK. Levels of androgens and estrogens in jugular blood as compared to those in blood entering and leaving the stallion testis. Abstract 330. Presented at the 69th Annual Meeting of the American Society of Animal Science, 1977.

Amann RP, Bowen JM, Pickett BW, Berndtson WE, Stashak TS, Voss JL. Reproductive function in stallions treated with cambendazole. J Am Vet Med Assoc 1977; 170:730–732.

Bedrak E, Samuels LT. Steroid biosynthesis by the equine testis. Endocrinology 1969; 85:1186–1195.

Corvol P, Bardin CW. Species distribution of testosterone-binding globulin. Biol Reprod 1973; 8:277–282.

deJong FH, Hey AH, van der Molen HJ. Effect of gonadotrophins on the secretion of oestradiol-17 and testosterone by the rat testis. J Endocrinol 1973; 57:277–284.

Enbergs H, Küpper G, Sommer H. Testosteronkonzentration in Serum von Warmblut-Zuchthengsten unter normalen und Belastungsbedingungen. Zuchthygiene 1977; 12:49–57.

Free MJ. Blood supply to the testis and its role in local exchange and transport of hormones. In: Johnson AD, Gomes, WR, eds. The testis. Vol 4. New York: Academic Press, 1977; 39–90.

Ganjam VK. Simultaneous ultramicroanalysis of both 17-keto and 17β-hydroxy androgens in biological fluids. Steroids 1976; 28:631–647.

Ganjam VK. Episodic nature of the Δ⁴-ene and Δ⁵-ene steroidogenic pathways and their relationship to the adreno-gonadal axis in stallions. J Reprod Fertil 1979; Suppl 27:67–71.

Ganjam VK, Amann RP. Steroids in fluids and sperm entering and leaving the bovine epididymis, epididymal tissue and accessory sex gland secretions. Endocrinology 1976; 99:1618–1630.

Ganjam VK, Amann RP. Effects of hCG-treatment on steroidogenesis in the stallion testis. Abstract 50. Presented at the Fourth Annual Meeting of the American Society of Andrology, 1979.

Ganjam VK, Kenney RM. Androgens and oestrogens in normal and cryptorchid stallions. J Reprod Fertil 1975; Suppl 23:67–73.

Ippensen E, Klug-Simon C, Klug E. Der verlauf der Blutgefässe von Hoden des Pferds in Hinblick auf ein Biopsiemöglichkeit. Zuchthygiene 1972; 7:35–45.

Kelch RP, Jenner MR, Weinstein R, Kaplan SL, Grumbach MM. Estradiol and testosterone secretion by human, simian and canine testes in males with hypogonadism and in male pseudohermaphrodites with the feminizing testes syndrome. J Clin Invest 1972; 51:824–830.

Kirkpatrick JF, Vail R, Devous S, Schwend S, Baker CB, Wiesner L. Diurnal variation of plasma testosterone in wild stallions. Biol Reprod 1976; 15:98–101.

Lindner HR. Androgens and related compounds in the spermatic vein blood of domestic animals. IV. Testicular androgens in the ram, boar and stallion. J Endocrinol 1961; 23:171–178.

Nyman MA, Geiger J, Goldzieher JW. Biosynthesis of estrogen by the perfused stallion testis. J Biol Chem 1959; 234:16–18.

Oh R, Tamaoki BI. Steroidogenesis in the equine testis. Acta Endocrinol 1970; 64:1–16.

Setchell BP. The mammalian testis. Ithaca: Cornell University Press, 1978; 109–180.

Sharma OP. Diurnal variations of plasma testosterone in stallions. Biol Reprod 1976; 15:158–162.

Squires EL, Amann RP, Hoyer J, Nett TM, Pickett BW. Effect of ejaculation on systemic levels of testosterone and LH in stallions. Abstract 523. Presented at the 69th Annual Meeting of the American Society of Animal Science, 1977.

Squires EL, Amann RP, Pickett BW, Berndtson WE, Shideler RK, Voss JL. Effect of fenbendozole on reproductive function in stallions. Theriogenology 1978; 9:447–455.

Thompson DL, Jr, Pickett BW, Berndtson WE, Voss JL, Nett TM. Reproductive physiology of the stallion. VIII. Artificial photoperiod, collection interval and seminal characteristics, sexual behavior and concentrations of LH and testosterone in serum. J Anim Sci 1977; 44:656–664.