

THE EFFECT OF YOHIMBINE ON PLASMA LEVELS OF T₃, T₄ AND CORTISOL IN XYLAZINE-IMMOBILIZED WHITE-TAILED DEER

GEORGE A. BUBENIK* and ROBERT D. BROWN†

*Department of Zoology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1. Telephone (519) 824-4120, and †Caesar Kleberg Wildlife Research Institute, Texas A & I University, Kingsville, TX 78363, USA

(Received 7 April 1988)

Abstract—1. The effect of yohimbine (Y) on blood levels of thyroxine (T₄), triiodothyronine (T₃), and cortisol was investigated in 5 mature male white-tailed deer immobilized with xylazine hydrochloride (X).
2. T₄ levels were erratic in X-treated deer, but stabilized in the X- and Y-treated deer.
3. T₃ remained unchanged in both groups.
4. Cortisol levels have increased in X-treated deer, but declined in X- and Y-treated deer.
5. Yohimbine is a potent and safe antidote of X not affecting T₃ and T₄. Caution should be used in using R or Y in cortisol studies.

INTRODUCTION

For more than 15 years xylazine hydrochloride (X) (Rompun®) has been used as a safe and effective sedative and anaesthetic for deer species (Bauditz, 1972; Presnell *et al.*, 1973; Roughton, 1975; Bubenik, 1982). Since immobilization by X is used routinely for investigation of endocrine parameters, concerns have been raised whether this compound might influence blood hormonal levels. However, it soon became clear, that X does not significantly influence blood levels of thyroxine (T₄), triiodothyronine (T₃), calcitonin, parathormone, testosterone, androstenedione, growth hormone or prolactin (Faulkner *et al.*, 1979; Mautz *et al.*, 1980; Bubenik, 1982). The only exception reported has been cortisol, for which blood levels were found to increase after X administration (Chao *et al.*, 1984; Van Mourik and Stelmasiak, 1984).

Until recently, the recovery from X immobilization was a rather slow process, which could be facilitated only indirectly by central analeptic drugs such as doxapram or caffeine (Bubenik, 1982; MacKintosh and VanReenen, 1984). However, several years ago yohimbine hydrochloride (Y) was tested in deer and found to be a potent and safe antidote of X (Jessup *et al.*, 1983; Hsu and Shulaw, 1984; Mech *et al.*, 1985; Renecker and Olsen, 1985; Van Der Eems and Brown, 1986).

As the popularity of Y in deer endocrine research increased, occasionally this drug had to be used in emergencies, such as during serial blood sampling which often lasted for several hours. Because it was not determined whether Y influence hormonal parameters, we devised a study which investigated the blood levels of hormones and enzymes in X immobilized deer treated subsequently with Y. In this article we report the effect of Y on plasma levels of T₃, T₄ and cortisol.

MATERIALS AND METHODS

Animals and experimental procedure

All experiments were performed in the deer facility of

Caesar Kleberg Wildlife Research Institute, Texas A & I University in Kingsville, Texas, USA (latitude 27:3°N). Five mature, pen-raised, adult male white-tailed deer (*Odocoileus virginianus*) were housed in 5 × 5 m, covered individual pens and fed a complete pelleted diet and fresh water *ad libitum*.

In the first experiment performed on 2 July the deer were immobilized with X (Rompun® 100 mg/ml; Haver-Lockhard Lab., Shawnee, KS 66201, USA) using Telinject blow-dart (Telinject USA, Inc., Newhall, CA 91321, USA). The doses of X (3.3; 3.3; 3.6; 4.4 and 7.2 mg/kg) were calculated according to individual sensitivity of animals to achieve full lateral recumbency. All bucks were fully immobilized except No. 38, which received the highest dose, was heavily sedated but remained standing. After immobilization the animals were sampled from the jugular vein into heparinized syringes. After taking 3 samples 15 min apart 10 ml of saline was injected intravenously (i.v.) and another 5 samples were taken at the same time interval.

In the second experiment, performed on 14 July, the procedure was repeated, except that yohimbine hydrochloride (5 mg/ml; Sigma Chemical Co., St Louis, MO; 63178, USA) was injected i.v. in a dose of 0.3 mg/kg instead of saline. Both experiments were performed between 07:30 and 10:00 hr.

Blood samples were placed on ice immediately and after each experiment they were centrifuged and plasma frozen for later assays.

Radioimmunoassays

Plasma total triiodothyronine (T₃), thyroxine (T₄) and cortisol levels were measured in duplicate samples using highly specific radioimmunoassays (RIA) (RIA Inc. Scarborough, Ontario, Canada). Intra-assay and inter-assay variability was less than 10%, and good correlations were found between sample volume and measured hormone levels for all assays. The lowest detectable levels were as follows: T₃, 12 ng/dl; T₄, 0.2 µg/dl, and cortisol, 0.5 µg/dl. The percentage binding at T₃ was 43.2 ± 0.2 (mean ± SE) and for T₄ 59.8 ± 1.7. All hormone assays were carried out within 3 months of the sample collection.

Statistical analysis

The first 3 pre-treatment samples were averaged for a pretreatment value and are depicted in Table 1 as the period 1. Each subsequent post-treatment sampling (samples 4-8) were treated as a separate period (2-6). As there are often

Table 1. Mean values (\bar{X}) and standard deviations (SD) for plasma hormone levels in 5 adult white-tailed deer treated with xylazine and saline or xylazine and yohimbine and sampled every 15 min

Parameter period	Xylazine + saline		Xylazine + yohimbine		P^*
	\bar{X}	SD	\bar{X}	SD	
<i>Cortisol</i>					
1†	8.51A	5.9	5.51AB‡	4.2	0.1252
2	13.48BC	6.3	7.18A	5.0	0.0363
3	14.98B	6.7	4.00BC	4.1	0.0293
4	14.98B	6.9	3.56BC	4.2	0.0109
5	13.50BC	4.5	2.59C	2.7	0.0053
6	10.43AC	5.5	1.97C	1.6	0.0299
T_3					
1	180.80A	46.1	146.91A	62.1	0.1301
2	185.80A	50.9	157.00A	80.3	0.2476
3	199.80A	36.2	158.40A	72.9	0.1298
4	221.40A	76.1	153.80A	60.8	0.0628
5	245.20A	77.2	147.68A	41.4	0.0068
6	224.60A	118.4	147.64A	54.3	0.1200
T_4					
1	14.40A	1.7	15.77A	1.6	0.0831
2	15.96B	2.0	16.42A	2.5	0.6395
3	14.90AC	1.4	16.72A	1.9	0.0282
4	14.68A	1.7	15.88A	2.0	0.0813
5	14.70A	1.7	17.12A	2.1	0.0517
6	15.62BC	1.3	15.92A	0.9	0.7320

*Paired t -test, xylazine + yohimbine vs xylazine + saline.

†Period 1 is mean of 3 samples ($N = 15$) after immobilization and prior to injection of saline or yohimbine.

‡Period means, within treatments and parameters, with the same capital letter are similar ($P > 0.05$), as established by analysis of variance (randomized complete block) and Fisher's least significant difference test (Gill, 1978).

individual responses of deer during experimental conditions, each animal was compared with itself. The mean shown represents the average of the difference between X-Saline (X + S) and X - Y (X + Y) values. Paired t -tests were used to compare values of cortisol (C), T_3 and T_4 for each of the 6 periods. An ANOVA (randomized complete block) and Fisher's LSD test were used within each treatment (X + S and X + Y) to compare periods (Gill, 1978).

RESULTS

Except for deer No. 38, all bucks were completely sedated by X, and the first few blood samples were taken in lateral recumbency. Injection of saline did not change that condition, but with passing time the deer switched to sternal recumbency. Administration of Y induced a rapid recovery (deer attempted to stand up and walk within 1-5 min). One deer (No. 38) had to be manually restrained to take blood samples.

For T_3 , (Fig. 1) there was no difference ($P > 0.05$) between X + Y and X + S groups except for period 5. Within group comparisons indicated that T_3 remained stable throughout sampling in both groups (Table 1).

For T_4 , (Fig. 2) there was also no difference ($P > 0.05$) between the X + Y and X + S groups, except for the period 3. For the X + Y group, the T_4 levels remained constant ($P > 0.05$) over all samples. For the X + S group, the T_4 values first increased, then decreased and finally increased again (Table 1).

For cortisol (C), (Fig. 3) the pre-treatment values were not different between groups ($P > 0.05$), but for periods 2-6, the X + S values were significantly greater than the X + Y values (Table 1). Within group comparisons indicated that the X + S values increased in periods 2-5, then declined in period 6.

Cortisol levels in the X + Y group tended to decline with time.

DISCUSSION

Yohimbine hydrochloride, an α_2 -adreno-receptor blocker (Goldberg and Robertson, 1983)

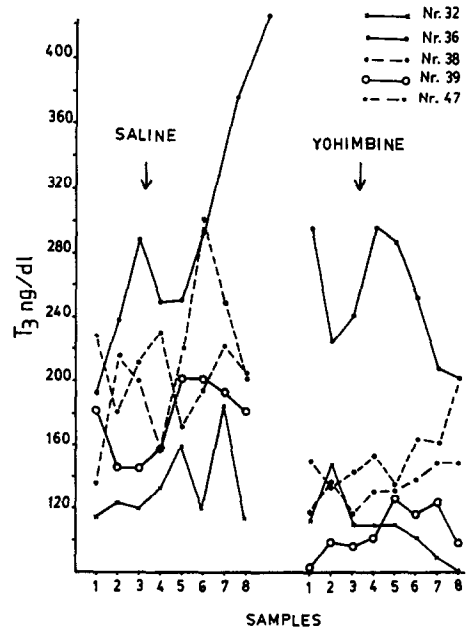


Fig. 1. Blood levels of triiodothyronine (T_3) in 5 X-immobilized white-tailed bucks treated intravenously (arrow) with either saline (S) or 0.3 mg/kg yohimbine (Y). Samples taken 15 min apart.

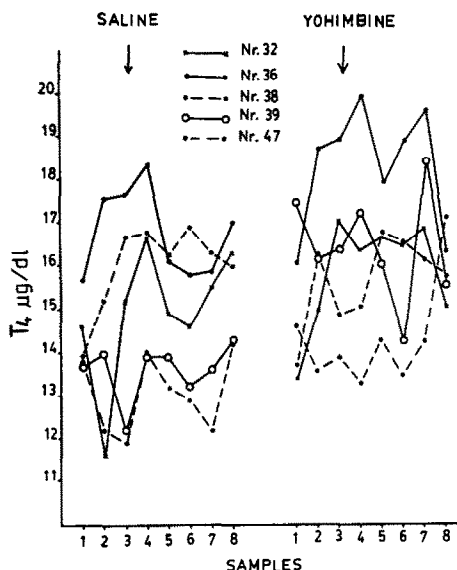


Fig. 2. Blood levels of thyroxine (T₄) in 5 xylazine-immobilized white-tailed bucks treated intravenously (arrow) with either saline (S) or 0.3 mg/kg yohimbine (Y). Samples taken 15 min apart.

was found to be a safe and effective antidote of X in red deer (*Cervus elaphus*) (MacKintosh and VanReenen, 1984; MacKelvey and Simpson, 1985), mule deer (*Odocoileus hemionus*) (Jessup *et al.*, 1983; Renecker and Olson, 1985) and white-tailed deer (Hsu and Shulaw, 1984; Renecker and Olson, 1985; Mech *et al.*, 1985), especially when fast recovery was required. In our experiments, the recovery time was not measured, but generally was in the range reported by other investigators (Mech *et al.*, 1985).

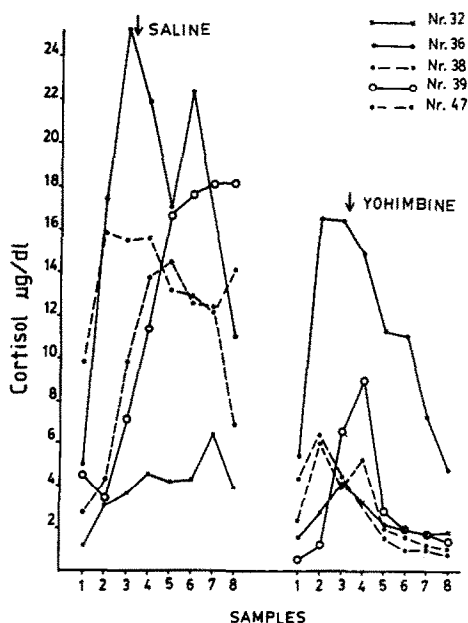


Fig. 3. Blood levels of cortisol in 5 xylazine-immobilized white-tailed bucks treated intravenously (arrow) with either saline (S) or 0.3 mg/kg yohimbine (Y). Samples taken 15 min apart.

The Y dose chosen for our study (0.3 mg/kg), was higher than that used by Jessup and co-workers (1983) (0.125 mg/kg) or Hsu and Shulaw (1984) (0.1 mg/kg). It has been reported that a higher dose than 0.1 mg/kg reduces recovery time to only several minutes (MacKintosh and VanReenen, 1984; Brown, personal communication). However it was also observed that a threshold dose of about 0.26 mg/kg may exist for white-tailed deer, beyond that not decrease of recovery time can be achieved (Mech *et al.*, 1985). Speed of recovery is essential in emergencies during serial blood sampling. On the other hand the fast recovery process might influence levels of blood hormones.

Generally, the levels of thyroid hormones were not substantially influenced by Y treatment. The levels of T₄ in white-tailed deer are maintained in a relatively narrow range (Bubenik *et al.*, 1977, 1983; Bubenik and Bubenik, 1978; Bubenik and Smith, 1986). During our serial sampling, values of T₄ in X + S group exhibited somewhat erratic fluctuations, which were eliminated in the Y treated group (Fig. 2).

Unlike the stable seasonal levels of T₄ in white-tailed deer (Bubenik, 1986), the levels of T₃ are reported to have pronounced seasonal variations (Bubenik and Leatherland, 1984; Bubenik *et al.*, 1986). Blood concentrations of T₃ also increase in response to intramuscular administration of T₄ (Bubenik and Smith, 1986), and in white-tailed deer investigated for circadian variation during one yearly cycle, T₃ exhibited the largest 1 hr variation of 7 hormones measured (Bubenik *et al.*, 1983). So far, no circadian rhythm of T₄, T₃ or cortisol has been detected in deer (Bubenik *et al.*, 1983; Van Mourik and Stelmasiak, 1984). In the present experiment the fluctuation of T₃ levels were not statistically significant during the 2 hr of sampling, and yohimbine had no effect on T₃ levels (Fig. 1).

Blood levels of glucocorticoids have been used as an indicator of response to stress (Panaretto and Vickery, 1972; Baldwin *et al.*, 1974). The blood levels of C in deer are generally low as compared to some other mammals (Brown and Martin, 1974), and an increase during blood sampling varies tremendously according to the character and the level of habituation of individual deer (Bubenik, 1982; Bubenik, 1986). In this experiment the concentration of C (Fig. 3) reached in the third sample of the deer No. 36 in the X + S group was very close to the maximum levels observed in deer treated with 40 I.U. of ACTH (Bubenik, 1986). That the highest values (over 25 µg/dl) observed in deer No. 36 were almost 4 times higher than maximal levels in animal No. 32 is fairly typical of individual sensitivity of deer to stress of sampling (Bubenik, 1986).

The pre-treatment values of C were not different ($P > 0.05$) between the X + S and X + Y groups, but post-treatment concentrations were significantly different in all periods. This indicates that Y reduced the increase in C observed in the X + S deer. In all but one deer (the most excitable No. 36) the cortisol concentrations returned to basal levels (between 1 and 3 µg/dl) within 15 min after Y administration.

It can be concluded that Y does not alter the plasma concentration of T₃ or T₄ during serial, long-term sampling in X immobilized deer. It appears

that Y stabilizes fluctuating levels of T_4 and significantly reduces the elevation of C levels found in X treated deer. The use of Y can therefore be recommended as a safe X antidote in studies investigating thyroid hormone in deer. Alternatively, the significant decline in C levels 30 min after Y administration might limit the use of this drug in corticoid studies.

Acknowledgements—The authors would like to thank Tom W. Dailey and Janice Kidwell for their skillful technical help, as well as to Ralph Bingham and Nancy E. Koerth for their help with the statistical evaluation.

REFERENCES

- Baldwin D. L. M., Colombo J. A. and Sawyer C. H. (1974) Plasma prolactin, LH and corticosterone in rats exposed to a novel environment. *Am. J. Physiol.* **226**, 1366–1369.
- Bauditz R. (1972) Sedation, immobilization and anesthesia with Rompun® in captive and free-living wild animals. *Vet. med. Rev.* **9**, 204–226.
- Brown G. M. and Martin J. B. (1974) Corticosterone, prolactin and growth hormone responses to handling and a new environment in the rat. *Psychosom. Med.* **36**, 241–247.
- Bubenik G. A. (1982) Chemical immobilization of captive white-tailed deer and the use of automatic blood samplers. In (N. Amer. Symposium): *Chemical Immobilization of Wildlife* (Edited by Nielsen L., Haigh J. C. and Fowler M. E.), pp. 334–354. Wisconsin Humane Soc., Milwaukee, WI, USA.
- Bubenik G. A. (1986) Regulation of seasonal endocrine rhythms in male boreal cervids. In *Endocrine regulations as adaptation mechanisms to the environment* (Edited by Assenmacher I. and Boissin J.), pp. 461–474. CNRS, Paris.
- Bubenik G. A. and Bubenik A. B. (1978) Thyroxine levels in male and female deer (*Odocoileus virginianus*). *Can. J. Physiol. Pharmac.* **56**, 945–949.
- Bubenik G. A. and Leatherland J. F. (1984) Seasonal levels of cortisol and thyroid hormones in intact and castrated mature male white-tailed deer. *Can. J. Zool.* **62**, 783–787.
- Bubenik G. A. and Smith J. H. (1986) The effect of thyroxine (T_4) administration on plasma levels of triiodothyronine (T_3) and T_4 in male white-tailed deer. *Comp. Biochem. Physiol.* **83A**, 185–187.
- Bubenik G. A., Bubenik A. B., Trenkle A., Sirek A., Wilson D. A. and Brown G. M. (1977) Short-term changes in plasma concentration of cortisol growth hormone and insulin during the annual cycle of a male white-tailed deer (*Odocoileus virginianus*). *Comp. Biochem. Physiol.* **58A**, 387–391.
- Bubenik G. A., Bubenik A. B., Schams D. and Leatherland J. F. (1984) Circadian and circannual rhythms of LH, FSH, testosterone, prolactin, cortisol, T_3 and T_4 in plasma of mature white-tailed deer. *Comp. Biochem. Physiol.* **76A**, 37–45.
- Bubenik G. A., Smith P. S. and Schams D. (1986) The effect of orally administered melatonin on the seasonality of deer pelage exchange, antler development, LH, FSH, prolactin, testosterone, T_3 , T_4 , cortisol and alkaline phosphatase. *J. Pineal Res.* **3**, 331–349.
- Chao C. C., Brown R. D. and Deftos T. T. (1984) Effects of xylazine immobilization on biochemical and endocrine values in white-tailed deer. *J. Wildl. Dis.* **20**, 328–332.
- Faulkner L. W., Zamora L., Jimenez M. and Brown R. D. (1979) The effect of xylazine hydrochloride on some blood parameters in white-tailed deer. NIH-MBS Symposium, Atlanta, GA. (Abstract).
- Gill J. C. (1978) *Design and Analysis of Experiments in the Animal and Medical Sciences*, Vol. I. Iowa State University Press, Ames.
- Goldberg M. R. and Robertson D. (1983) Yohimbine: a pharmacological probe for study of the alpha₂-adreno-receptor. *Pharmac. Rev.* **35**, 143–180.
- Hsu W. J. and Shulaw W. L. (1984) Effect of yohimbine on xylazine induced immobilization in white-tailed deer. *J. Am. vet. med. Ass.* **185**, 1301–1303.
- Jessup D. A., Clark W. E., Gullett P. A. and Jones K. R. (1983) Immobilization of mule deer with ketamine and xylazine and reversal of immobilization with yohimbine. *J. Am. vet. Med. Ass.* **183**, 1339–1340.
- McKelvey W. A. C. and Simpson C. A. (1985) Reversal of the effects of xylazine and xylazine/ketamine in red deer. *Vet. Rec.* **117**, 362–363.
- MacKintosh C. G. and Van Reenen G. (1984) Comparison of yohimbine, 4-aminopyridine and doxapram antagonism of xylazine sedation in deer (*Cervus elaphus*). *N.Z. Vet. J.* **32**, 181–184.
- Mautz W. W., Seal U. S. and Boardman C. B. (1980) Blood serum analyses of chemically and physically restrained white-tailed deer. *J. Wildl. Mgmt* **44**, 343–351.
- Mech L. D., Delguidice G. D., Karns P. D. and Seal U. S. (1985) Yohimbine hydrochloride as an antagonist to xylazine hydrochloride immobilization of white-tailed deer. *J. Wildl. Dis.* **21**, 405–410.
- Panaretto B. A. and Vickery M. R. (1972) The distribution of cortisol and its rate of turnover in normal and cold-stressed shorn sheep. *J. Endocr.* **55**, 519–531.
- Presnell K. R., Presidente P. J. A. and Rapley W. A. (1973) Combination of etorphine and xylazine in captive white-tailed deer. I. Sedative and immobilization properties. *J. Wildl. Dis.* **9**, 336–341.
- Roughton R. D. (1975) Xylazine as an immobilizing agent for captive white-tailed deer. *J. Am. vet. Med. Ass.* **167**, 574–576.
- Renecker L. A. and Olsen C. D. (1985) Use of yohimbine and 4-aminopyridine to antagonize xylazine-induced immobilization in North American Cervidae. *J. Am. vet. Med. Ass.* **187**, 1199–1201.
- Van Der Eems K. and Brown R. D. (1986) Effect of caffeine sodium benzoate, ketamine hydrochloride, and yohimbine hydrochloride on xylazine hydrochloride-induced anorexia in white-tailed deer. *J. Wildl. Dis.* **22**, 403–406.
- Van Mourik S. and Stelmasiak T. (1984) The effect of immobilizing drugs on adrenal responsiveness to ACTH in Rusa deer. *Comp. Biochem. Physiol.* **79A**, 581–584.