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EFFECTS OF XYLAZINE IMMOBILIZATION ON BIOCHEMICAL AND ENDOCRINE VALUES IN WHITE-TAILED DEER

Chun Chin Chao,[1] Robert D. Brown,[1] and Leonard J. Deftos[2]

ABSTRACT: The effect of xylazine hydrochloride on biochemical and endocrine parameters in plasma was examined in adult white-tailed deer (*Odocoileus virginianus* (Zimmermann)). In the first experiment, seven animals were injected intramuscularly via a blowgun dart with 0.65 mg/kg xylazine (100 mg/ml) and were bled 10, 20, 30, and 60 min post-injection. In the second experiment, eight animals were manually restrained for the first blood sampling and then injected manually and bled as before. Plasma calcium (Ca), inorganic phosphorus (P), and alkaline phosphatase (AP) were measured spectrophotometrically. Plasma parathyroid hormone (PTH), calcitonin (CT), thyroxine (T_4), triiodothyronine (T_3), and cortisol were measured by radioimmunoassay. Plasma PTH, CT, T_4, T_3, and AP activity did not differ ($P > 0.05$) during the 1 hr period studied in either experiment. Plasma Ca and P decreased significantly ($P < 0.05$) in the second experiment, whereas cortisol levels increased significantly ($P < 0.05$) 10 min post-injection in both experiments. The results may have been due to a drug effect or a combined drug and stress effect. It is suggested that xylazine may be safely used as an anesthetic in measuring PTH, CT, T_4 and T_3, and plasma AP up to 60 min post-injection in deer. Caution should be taken in using xylazine as an anesthetic to study adrenocortical function.

INTRODUCTION

Serum biochemical and endocrine measurements have received increased attention as a useful means of evaluating animal condition and health. Extensive studies are available on the effects of chemical immobilization on many hematological values in white-tailed deer (Presidente et al. 1973; Mautz et al., 1980; Kocan et al., 1981). Individual animal variations and sample handling techniques may have directly or indirectly affected the results of these studies. Consequently, increased sample size and a standardized technique are necessary to improve interpretation of results.

Xylazine hydrochloride has been widely used in both penned and free-ranging whitetails (Presnell et al., 1973; Roughton, 1975). There have been few studies of the effect of xylazine on circulating endocrine values (Faulkner et al., 1979). The purpose of this study was to evaluate the effect of xylazine on plasma biochemical and endocrine values in white-tailed deer.

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MATERIALS AND METHODS

This study was conducted at the Texas A&I University Wildlife Research Facility, 3 km north of Kingsville, Texas. Four male and four non-pregnant, female adult (3-yr-old), penraised, white-tailed deer (45–65 kg) were individually housed in 5 × 5-m covered pens and fed a complete pelleted diet (P&M Products, P.O. Box 7146, San Antonio, Texas 78207, USA) and fresh water ad libitum. The experiments were done in April of 1982 and 1983, respectively. Seasonal effects were avoided by choosing the same month in different years. Animals were fasted overnight before each experiment to avoid a possible feeding effect on blood values (Perault-Staub et al., 1975).

In the first experiment, seven animals (3 males and 4 females) were anesthetized with 0.65 mg/kg xylazine hydrochloride (Rompun, Cutter Laboratories, Bayvet Division, Shawnee, Kansas 66201, USA) administered by blow gun (Telinject USA, Inc., Encino, California 91436, USA). Blood samples were then taken via jugular venipuncture into 10-ml NH_4 heparinized syringes 10, 20, 30, and 60 min post-injection. In order to avoid stress, no initial samples were taken prior to or concurrent with injection of xylazine in this experiment.

In the second experiment, eight animals (4 males and 4 females) were manually restrained,

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bled via jugular venipuncture, and manually injected with xylazine. Blood samples were similarly withdrawn 10, 20, 30, and 60 min after injection. Induction time was 6–10 min after administration of the xylazine in both experiments. Blood samples were centrifuged, and the plasma was separated and stored at −40 C for further analysis.

Plasma total calcium (Ca) was determined in duplicate samples by atomic absorption spectrophotometry (AA-1475, Varion, 505 Julie Rivers Road, Sugar Land, Texas 77878, USA). Plasma inorganic phosphorus (P) and alkaline phosphatase activity (AP) were determined on duplicate samples spectrophotometrically with colorimetric kits (Hycel Products, P.O. Box 36329, Houston, Texas 77036, USA). Plasma calcitonin (CT) was measured on duplicate samples, using a bovine radioimmunoassay (Deftos et al., 1972) that was tested previously in deer with a $CaCl_2$ infusion. An infusion of 5% $CaCl_2$ at a rate of 3 mg/kg/min for 20 min in three deer resulted in a five- to eight-fold increase in CT levels, thus indicating the usefulness of the assay in deer (Chao, 1983). Guinea pig anti-bovine CT and rabbit anti-guinea pig antisera were used as antibodies. The sensitivity of the assay was 1 pg/ml, and the intraassay coefficient of variance (CV) was 10%. Plasma PTH was measured using a bovine radioimmunoassay (Iso-Tex Diagnostics, Box 909, Friendswood, Texas 77546, USA). The PTH assay was tested by infusion of 8% EDTA at a dose of 2.16 mg/kg/min in four deer. The infusion caused a three- to four-fold measurable increase. Guinea pig anti-bovine PTH and goat anti-guinea pig antisera were used as the first and second antibodies. ^{125}I-bPTH served as the tracer. The sensitivity of the assay was 0.1 ng/ml, and the intraassay CV was 6.09%. Plasma cortisol was measured by a radioimmunoassay from Pantex (1737 21st St., Santa Monica, California 90404, USA). Rabbit anti-human and goat anti-rabbit antisera were used as the first and second antibodies. ^{125}I was used as the tracer. The sensitivity of the assay was 50 pg/dl, and the intraassay CV was 10.4%. Plasma total thyroxine (T_4) and triiodothyronine (T_3) were measured by radioimmunoassays from Wien Labs (Box 227, Succasunna, New Jersey 07876, USA). Rabbit antisera, ^{125}I labeled antigens, and a charcoal separation technique were used in both assays. The intraassay CV's for T_4 and T_3 were 3.8 and 6%, respectively. The sensitivity was 0.1 μg/dl for T_4 and 15 ng/dl for T_3.

A split-plot design (Gill, 1978) for repeated measurements was analyzed using the Statistical Analysis System (SAS) software package (Ray, 1982). A Ryan-Einot-Gabriel-Welsch multiple *F*-test was used for mean comparisons among bleeding periods.

RESULTS

Table 1 presents mean plasma biochemical and endocrine values for each bleeding period for both experiments. An analysis of variance for a split-plot design indicated that there were no sex differences ($P > 0.05$) for any of the parameters examined except T_3 and P in the first experiment, where a significant ($P < 0.05$) interaction between sex and time was found. Plasma T_3 and P were then tested within sexes, and the results indicated a lack of significant ($P < 0.05$) differences between times of bleeding. The subsequent analysis of the rest of the parameters was based on pooled data for males and females.

In the first experiment, significant ($P < 0.05$) differences between time of bleeding were found only in plasma cortisol (Table 1). The peak in plasma cortisol (5.88 μg/dl) was found 30 min post-injection of xylazine. However, values increased from 10–30 min post-injection. The values declined thereafter. In the second experiment, significant ($P < 0.05$) differences were found in plasma Ca, P, and cortisol, but not in PTH, CT, T_4, T_3, or AP pre- and post-injection of xylazine. Plasma Ca was significantly ($P < 0.05$) higher before the injection. Plasma P decreased significantly ($P < 0.05$) 10 min after administration of xylazine. Plasma cortisol increased significantly ($P < 0.05$) post-injection, but there was no significant difference ($P > 0.05$) between any of the post-injection values.

DISCUSSION

In these adult deer, the lack of a sex effect on T_4 and cortisol was consistent with our previous study in yearling deer (Chao and Brown, 1984). However, male fawns have higher T_4 levels than female fawns (Brown et al., 1983b). Serum AP is

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TABLE 1. Mean (±SE) plasma biochemical and endocrine values pre- and post-injection of xylazine hydrochloride for seven (3 males and 4 females) deer in experiment 1 and eight (4 males and 4 females) deer in experiment 2.

	Pre-injection		Minutes post-injection[a,b]							
			10		20		30		60	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Experiment 1										
Ca (mg/dl)			8.79	0.06	8.66	0.25	8.87	0.22	8.81	0.16
P (mg/dl)			7.64	0.41	7.47	0.40	7.46	0.41	7.54	0.49
AP (IU/liter)			25.78	0.71	25.87	1.31	26.38	0.98	25.14	2.88
PTH (ng/ml)			1.17	0.17	1.11	0.20	1.22	0.18	1.24	0.14
CT (ng/ml)			1.33	0.29	0.73	0.07	1.06	0.38	1.00	0.08
T_4 (μg/dl)			8.23	0.12	7.87	0.15	8.47	0.24	8.24	0.09
T_3 (ng/dl)			97.43	5.28	97.44	6.28	97.44	5.80	91.16	2.87
Cort (μg/dl)			1.43[c]	0.10	3.32[d]	0.57	5.88[e]	0.92	3.15[d]	0.85
Experiment 2										
Ca (mg/dl)	9.53[c]	0.19	8.81[d]	0.16	8.47[e]	0.19	8.78[d]	0.18[d,e]	8.75[d,e]	0.22
P (mg/dl)	7.94[c]	0.43	7.13[d]	0.49	6.15[e]	0.45	6.46[d,e]	0.46	6.23[e]	0.54
AP (IU/liter)	22.23	1.65	22.21	2.17	21.16	2.52	22.72	1.72	21.85	1.92
PTH (ng/ml)	1.73	0.32	1.59	0.15	1.55	0.14	1.63	0.12	2.00	0.26
CT (ng/ml)	1.75	0.39	1.47	0.33	1.38	0.28	1.41	0.27	1.35	0.22
T_4 (μg/dl)	8.16	0.22	8.45	0.37	8.72	0.22	8.14	0.22	8.67	0.15
T_3 (ng/dl)	93.30	13.04	89.91	18.23	88.71	13.30	87.95	9.52	82.54	10.15
Cort (μg/dl)	1.32[c]	0.11	2.80[d]	0.20	3.48[d]	0.20	3.65[d]	0.43	2.94[d]	0.53

[a] 0.65 mg/kg xylazine (100 mg/ml), injected IM.

[b] Means with different superscripts (c,d,e) in a row are significantly different ($P < 0.05$) by the Ryan-Einst-Gabriel-Welsch multiple F-test.

affected by seasons (Brown et al., 1983a), but our study was done in April when AP activity in males was low and similar to that of females. Lack of a sex effect on plasma PTH and CT is inconsistent with findings in cattle (Deftos et al., 1972) and humans (Deftos et al., 1980). Again, our findings may have been due to the time of year, a period when whitetails are quiescent generally.

Mautz et al. (1981) reported that xylazine had no short-term (10–60-min) or long-term (1–4-day) effect on blood T_4 or AP. Our results were in agreement with theirs and a study by Faulkner et al. (1979). However, in our first experiment, there was a significant ($P < 0.05$) increase in cortisol values 20 min after injection which peaked 30 min after injection. Since injection via the blow-dart seemed not to disturb the deer and since the deer were induced before the first blood sampling, the latter increase in cortisol was probably due to the drug itself. The lack of a significant change in plasma Ca and P 10–60 min post-injection was consistent with a previous study (Faulkner et al., 1979).

In the second experiment, the significant ($P < 0.05$) decrease in plasma Ca and P following administration of xylazine indicated either a stress or drug effect. However, since the first experiment ruled out the possible drug effect on Ca and P changes, this was probably due to the stress caused by manual restraint. The lowered value could be due to increased plasma volume (Seal et al., 1972; Presidente et al., 1973; Karns and Crichton, 1978; Wesson et al., 1979). This hypothesis was also supported by lack of a significant ($P > 0.05$)

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change in peripheral PTH and CT, which are sensitive regulating factors in response to peripheral ionized Ca changes (Sherwood et al., 1968). The lowered Ca and P levels 20 min post-injection of xylazine in the second experiment are comparable with other reports (Presidente et al., 1973; Karns and Crichton, 1978).

In the second experiment, cortisol increased significantly ($P < 0.05$) 10 min post-injection and peaked at 30 min. It is not possible to determine whether the increase was due to the drug or stress. Since the 10 min value in the first experiment was similar to the pre-injection value in the second experiment, the elevated values in the latter experiment were due probably to a combination of the stress of manual restraints and drug induction (Bubenik, 1982). Injection of 10 mg xylazine in Rusa deer by Van Mourik and Stelmasiak (1984) resulted in only a small elevation of cortisol levels 10 min post-injection. However, Presidente et al. (1973) found that when immobilization involved struggling or trauma, chemical changes of considerable magnitude were common.

In conclusion, xylazine may be used as an anesthetic when measuring hormones such as T_3, T_4, PTH, and CT, and blood AP activity up to 60 min post-injection. Blood Ca and P from samples collected 10–60 min post-injection of xylazine are consistent if no handling stress is involved. While cortisol has been used as an indicator of stress in deer (Franzmann et al., 1975; Bubenik et al., 1977; Wesson et al., 1979), caution should be taken when using xylazine to study adrenocortical function in deer.

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