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ANTAGONISM OF XYLAZINE HYDROCHLORIDE WITH YOHIMBINE HYDROCHLORIDE AND 4-AMINOPYRIDINE IN CAPTIVE WAPITI

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ABSTRACT: Eight captive wapiti (*Cervus elaphus nelsoni*) were injected with xylazine hydrochloride on two occasions during March and April 1984. Animals were grouped into a modified Latin square design and were given either successive injections of yohimbine hydrochloride and 4-aminopyridine (4-AP) to antagonize the sedative effects of xylazine hydrochloride or permitted an unantagonized recovery. Induction times ranged from 3 to 26 min with excited and wild animals requiring a supplementary dose. Time until walking was significantly ($P < 0.005$) shorter in the group given successive injections (given i.v.) of the reversal drugs yohimbine hydrochloride (0.15 mg/kg) and 4-AP (0.30 mg/kg) than those animals during unantagonized recoveries. Marked increases in heart rate and respiratory rate were observed in animals within 3 min after successive injections of yohimbine hydrochloride and 4-AP. There was no occurrence of convulsions and animals did not relapse to profound sedation. Slight muscle tremors were observed in one animal which received a dose of 0.35 mg/kg of 4-AP. This drug combination can reduce markedly the duration of recovery from xylazine hydrochloride-induced sedation in wapiti.

INTRODUCTION

Xylazine hydrochloride is used widely for the chemical restraint of ungulates in biological research, surgical procedures and veterinary medicine. This non-narcotic drug can induce a sedative, analgesic and muscle relaxing effect by autonomic and central nervous system depression (Sagner et al., 1968; Schmidl, 1974). For the immobilization of wild and captive Cervidae, xylazine hydrochloride often has been used alone (Roughton, 1975; Jacobsen, 1983) or in combination with narcotics such as etorphine or fentanyl hydrochloride (Jessup et al., 1980; Hebert and McFetridge, 1981; Thorne, 1982), to reduce the time and excitation associated with the induction period (Harthoorn, 1976).

Wild ruminants are relatively sensitive to the effects of xylazine hydrochloride. Dosages of 0.44 to 5.30 mg/kg can induce recumbency in black-tailed deer (*Odocoileus hemionus columbianus*) for 1 to 6 hr (Jacobsen, 1983). Wapiti (*Cervus elaphus nelsoni*) have been immobilized for 1 to several hr with dosages of 0.80 to 3.90 mg/kg of xylazine hydrochloride (Anon., 1984). Fletcher (1974) observed xylazine hydrochloride hypersensitivity in an insular population of red deer (*Cervus elaphus*), noting that dosages of 3 to 4 mg/kg produced profound immobilization for periods of 6 to 9 hr. This prolonged recumbency increases the possibility of bloat, regurgitation, aspiration, hyperthermia, hypothermia or pressure damage to nerve and muscle tissues. Nonetheless, xylazine hydrochloride is a valuable drug for wildlife biologists in that it is readily procurable and relatively safe.

Recently, a new drug combination has been used experimentally as an antagonist for xylazine hydrochloride. Yohimbine hydrochloride is an alkaloid that blocks the release of noradrenalin in the central nervous system, thereby stopping the action of this neurotransmitter on the central alpha$_2$-receptors (Hsu, 1981). In turn, this may be the same adrenergic mechanism agonized by xylazine hydrochloride (Cronin et al., 1983). The drug 4-aminopyridine (4-AP) may enhance the release of acetylcholine (Andén and Leander, 1979; Foldes et al., 1982) and other neurotransmitters (Booth et al., 1982; Löffelholz and Weide, 1982). Either of these

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drugs will antagonize partially the effects of xylazine hydrochloride (Kitzman et al., 1982), or ketamine hydrochloride plus xylazine hydrochloride (Jessup et al., 1983). However, a mixture of 4-AP and yohimbine hydrochloride in the same syringe effectively reduced arousal and standing times from xylazine hydrochloride-induced sedation in cattle (Kitzman et al., 1982) and dogs (Hatch et al., 1982; Wallner et al., 1982), better than either drug administered separately. Successive injections of yohimbine hydrochloride and 4-AP have been used as reversal drugs for xylazine hydrochloride-induced immobilization of captive moose (*Alces alces*), mule deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*) (Renecker and Olsen, 1985). Yohimbine hydrochloride alone has been used as a reversal drug for xylazine hydrochloride-induced immobilization of white-tailed deer (Hsu and Shulaw, 1984) and red deer (MacKintosh and Van Reenen, 1984).

The objective of this study was to determine if xylazine hydrochloride-induced immobilization in wapiti could be effectively antagonized by successive injections of yohimbine hydrochloride and 4-AP.

MATERIALS AND METHODS

The experiment involved the use of eight adult wapiti: four males and four females. Animals were maintained in a 65 ha enclosure at the Ministik Wildlife Research Area on natural forage and a pelleted alfalfa-concentrate ration. Body weights averaged 271 ± 28 kg for bulls and 251 ± 7 kg for cows. Animals were paired according to sex, age, body weight (visually estimated for one wild animal) and behavior. Behavior of an animal was described as wild and unapproachable by humans, born in captivity but excitable or tractable. In March 1984, each animal of a designated pair was assigned randomly to a treatment group which involved either xylazine hydrochloride-induced immobilization followed by unantagonized recovery or xylazine hydrochloride-induced sedation followed by antagonist administration using a modified Latin square design (treatment × sex). A period of 6 to 7 days was allowed between treatments (2 days for one male). All animals were allowed to feed on native forage within the enclosure, but received no supplementary feed for 2 days prior to immobilization.

Xylazine hydrochloride administration

Animals were immobilized by an injection of xylazine hydrochloride (Rompun®, Bayvet Division, Miles Laboratories Ltd., Mississauga, Ontario L4W 2A1, Canada) at a concentration of 100 mg/ml solution. Xylazine hydrochloride was administered intramuscularly (i.m.) into the rump of all animals. Tractable and excitable wapiti were injected by hand syringe, in a large animal squeeze. Wild animals were injected with projectile syringes, fired from a powder-charged rifle (Cap-Chur Powder Projector, Palmer Chemical and Equipment Co., Inc., Douglasville, Georgia 30133, USA) or a CO_2-powered pistol (Cap-Chur CO_2 Pistol, Palmer Chemical and Equipment Co., Inc., Douglasville, Georgia 30133, USA). A dose of 0.65 mg/kg of xylazine hydrochloride was given in the initial injection to immobilize the wapiti. This dose was intermediate to the value used for wild wapiti (Jessup et al., 1980; Anon., 1984) and from previous drug records at the Ministik Wildlife Research Station. A supplemental dose of xylazine hydrochloride was supplied if the initial dose was inadequate. Animals were weighed prior to xylazine hydrochloride administration (body weight of one wild animal was estimated).

Reversal of xylazine hydrochloride

The experimental antagonists were yohimbine hydrochloride (Sigma Chemical Co., St. Louis, Missouri 63178, USA) and 4-AP (Sigma Chemical Co., St. Louis, Missouri 63178, USA). Dosage of the antagonist was based on previous research with cattle (Kitzman et al., 1982) and mule deer (Jessup et al., 1983). Immobilized wapiti, in the treatment group, were given successive intravenous (i.v.) injections of yohimbine hydrochloride (0.15 mg/kg; 0.5% w/v solution in distilled, deionized water) and 4-AP (0.30 mg/kg; 1.0% w/v solution in distilled, deionized water). The concentration of yohimbine hydrochloride used in this study was increased by a factor of five over that recommended by Kitzman et al. (1982) to decrease the volume required per injection. Because a >0.1% solution of yohimbine hydrochloride will precipitate in the syringe when mixed with the 4-AP solution (1%), the solutions were administered in consecutive injections using separate syringes.

Monitored parameters

Heart rates, respiratory rates and rectal temperatures were recorded for all animals during immobilization and recovery. Presence of jaw, orbital and ear reflexes were used as indications of immobilization. The intervals of events measured were: (a) induction time (time from injection of xylazine hydrochloride to the animals' inability to stand); (b) arousal time (time from injection of antagonists until laterally recumbent animals regained the ability to maintain a sternal posture, eructate and were alert and responded to noise or, in animals not given the antagonists, it was the time between complete immobilization and lateral recumbency until the animal could maintain a sternal posture, eructate, was alert and responded to noise); (c) walking time (time from injection of the antagonists until the animal stood and walked or, in animals not given the antagonist, the time from complete immobilization until the animals stood and walked); and (d) total recovery time (time from injection of antagonists until the animals were walking with a steady gait and would eat or, in animals not given the antagonists, the time from complete immobilization until the animals were walking with a steady gait and could eat).

RESULTS AND DISCUSSION

Immobilization

Each animal was immobilized for two treatment procedures. Between sexes, effective dosages of xylazine hydrochloride (Table 1) were not significantly different ($P > 0.05$) as indicated by a two-way analysis of variance (Steele and Torrie, 1980). Dosages ranged from 0.64 to 2.18 mg/kg with three wild, excited animals requiring supplementary injections and the largest volumes of xylazine hydrochloride for effective immobilization. A paired t-test (Steele and Torrie, 1980) indicated that volumes injected by projectile syringe into wild, male animals were significantly ($P < 0.001$) higher than dosages given by hand injection to tractable males.

Wapiti responded initially to an i.m. injection of xylazine hydrochloride with reduced movement and a drooping of the ears. Subsequently, a lowered head and excessive salivation were followed by sternal recumbency after an average of 8.4 ± 1.5 min. A maximum induction time of 26 min (Table 1) was recorded for one wild male which was probably the combined result of an increased level of excitement, rebounding of projectile syringes away from the contacted rump and leakage of immobilizing solution from the injection site. Similar problems were observed by Jacobsen (1983) while tranquilizing black-tailed deer.

The induction period did not vary significantly ($P > 0.05$) between treatments for either sex. Although induction times were greater for males, significant differences were not observed ($P > 0.05$), possibly because of the small sample size. Furthermore, differences between mean induction times of males given either a hand injection or an injection of xylazine hydrochloride with a projectile syringe were not significant ($P > 0.05$).

Recovery time

Unantagonized animals regained sternal recumbency in 3.8 ± 0.4 hr. Walking required an additional 0.5 hr (Table 1). The longest times until walking, for animals given a dose of 0.65 mg/kg of xylazine hydrochloride, corresponded with the shortest induction times. Generally, short induction times and prolonged immobilization were typical of the cows, all of which were tractable. Poor body condition and relative tractability may have been factors in producing this response in one male.

Arousal times of xylazine hydrochloride-induced immobilization in wapiti were shortened by successive injections of yohimbine hydrochloride and 4-AP. Animals attained the ability to maintain a sternal posture, eructate and respond to auditory stimuli in an average of 4 ± 2.1 min. Arousal was indicated first by eye movement and vocalization. Animals would lift their head, eructate, respond to auditory stimuli and salivate profusely for a short period. Similarly, walking times were reduced to between 1.9 and 47 min

TABLE 1. Comparison of mean induction and walking times of xylazine hydrochloride-induced immobilization of wapiti without drug reversal and after antagonism with successive injections of yohimbine hydrochloride and 4-AP.

			No antagonist administered		Reversal with yohimbine HCl and 4-AP[a]	
Group	*n*	Mean xylazine HCl dosage (mg/kg) (±SE)	Mean induction time (a) (min) (±SE)	Mean walking time (c) (min) (±SE)	Mean induction time (a) (min) (±SE)	Mean walking time (c) (min) (±SE)
Male	4	1.19 (0.23)	13.0 (4.6)	232.6 (55.1)	4.9 (0.1)	15.2 (10.7)
Female	4	0.68 (0.02)	6.1 (1.6)	270.0 (18.2)	9.8 (2.7)	12.4 (6.4)

[a] Mean time between immobilization and antagonist administration equalled 17.6 min (±3.6).

(Table 1). Similar walking times of 7.4 min were reported for cattle immobilized with xylazine hydrochloride (Kitzman et al., 1982) and 1 to 30 min for mule deer immobilized with a xylazine hydrochloride-ketamine hydrochloride mixture and reversed with yohimbine hydrochloride (Jessup et al., 1983). Four animals, which were administered the reversal combination, became ambulatory in less than 3 min. These times until walking were significantly ($P < 0.005$) shorter than those observed during unantagonized recovery (Table 2). After arousal, antagonized wapiti would regain tongue control, stand, urinate and walk for a short time before consuming feed and snow. Total recovery times were shortened an average of 2.5 hr when animals were antagonized with successive injections of yohimbine hydrochloride and 4-AP. This permitted animals to become ambulatory, feed and interact with other animals, safely, in an average of 56 min.

The dose of 0.15 mg/kg of yohimbine hydrochloride and 0.30 mg/kg of 4-AP appeared effective as a reversal agent for xylazine hydrochloride-induced sedation in wapiti. However, an increase in the dosage of 4-AP to 0.35 mg/kg resulted in slight, generalized muscle tremors in one animal. These spasms have been reported in cattle which received a similar dosage (Kitzman et al., 1982). Convulsions were not observed in these animals during the drug reversals. Animals did not relapse to profound immobilization.

Volumes of yohimbine hydrochloride and 4-AP, which were injected into the one animal not weighed, were determined from a conservative estimate of body weight at a dosage level of 0.15 mg/kg

TABLE 2. Analysis of variance for walking times of xylazine hydrochloride-induced immobilization of wapiti permitted an unantagonized recovery and animals reversed with successive injections of yohimbine hydrochloride and 4-AP.

Source of variance	Degrees of freedom	Sum of squares	Mean squares	*F*-value
Sex	1	1,237	1,237	0.367 NS[a]
Animal × sex	6	20,246	3,374	0.972 NS
Treatment	1	226,267	226,267	65.169[b]
Day	1	1,691	1,691	0.487 NS
Treatment × sex	1	4,680	4,680	1.348 NS
Error	5	17,362	3,472	
Total	15	271,483		

[a] NS = not significant at the 0.05 probability level.
[b] Significant at the 0.005 probability level.

TABLE 3. Effect of successive injections of yohimbine hydrochloride and 4-AP on mean respiratory rates and heart rates in wapiti immobilized with xylazine hydrochloride.

		Mean respiration rate (breaths/min)			Mean heart rate (beats/min)	
Group	n	During induction (±SE)	During immobilization (±SE)	After antagonist (±SE)	During immobilization (±SE)	After antagonist (±SE)
Male	4		13.4 (0.4)	25.3 (4.8)	30.0 (1.0)	48.0 (13.5)
Female	4	25.0 (1.5)	9.5 (0.3)	17.6 (4.1)	30.0 (0.5)	45.0 (3.3)

and 0.25 mg/kg, respectively. No adverse effects (muscle tremors or convulsions) were observed in this individual.

Environmental and physiological conditions

Differences ($P < 0.001$) were evident in mean rates of respiration of female wapiti between induction and immobilization periods (Table 3). Further, drug reversal of xylazine hydrochloride-immobilized wapiti produced significant changes in mean respiration (male, $P < 0.001$; female, $P < 0.05$) and heart (male, $P < 0.002$; female, $P < 0.001$) rates. Breathing and pulse rates increased an average of 10 breaths/min and 15.5 beats/min, respectively, within 3 min of the consecutive injections of yohimbine hydrochloride and 4-AP, similar results having been observed in dogs (Wallner et al., 1979). Both yohimbine hydrochloride and 4-AP appear to contribute positively towards this excitation of respiratory activity (Luckens and Malone, 1973; Agoston et al., 1982).

During immobilization, rectal temperatures averaged 38.5 ± 0.04 C for males and 38.2 ± 0.10 C for females. These values were recorded during ambient temperatures which ranged from −13 C to 6 C. A moist or snow-covered ground surface may have prevented elevations in rectal temperatures on days of warm air temperatures. Late afternoons or days with cloud cover were selected for immobilization using projectile syringes, thus, wapiti which ran or struggled during tranquilization did not overheat.

Successive injections of yohimbine hydrochloride and 4-AP can be used as an effective reversal drug combination for xylazine hydrochloride-induced immobilization of wapiti. The cost of these drugs, per immobilized animal (300 kg animal), was approximately $0.13 Canadian.

Excited animals may require large volumes of xylazine hydrochloride resulting in incomplete immobilization (Keep, 1979; Thorne, 1980); however, lengthy and profound sedation can occur in sensitive or very tractable animals at these high doses. Nevertheless, this antagonist combination can effectively antagonize the sedative and immobilizing actions of xylazine hydrochloride in wapiti.

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LITERATURE CITED

AGOSTON, S., D. R. A. UGES, AND R. L. SIA. 1982. Therapeutic applications of 4-aminopyridine in anesthesia. *In* Aminopyridines and Similarly Acting Drugs: Effects on Nerves, Muscles and Synapses, P. Lechat et al. (eds.). Pergamon Press, Toronto, Ontario, pp. 303–311.

ANDÉN, N. E., AND S. LEANDER. 1979. Effects of 4-aminopyridine on turnover of monoamines in the central nervous system of the rat. J. Neurol. Transm. 44: 1–12.

ANONYMOUS. 1985. Techniques for chemical restraint of Alberta wildlife. Technical Manual. Alberta Energy and Natural Resources, Fish and Wildlife Div., Edmonton, Alberta. In prep.

BOOTH, N. H., R. C. HATCH, AND L. M. CRAWFORD. 1982. Reversal of the neurolept-analgesic effect of droperidol-fentanyl in the dog by 4-aminopyridine and naloxone. Am. J. Vet. Res. 43: 1227–1231.

CRONIN, M. F., N. H. BOOTH, R. C. HATCH, AND J. BROWN. 1983. Acepromazine-xylazine combination in dogs: Antagonism with 4-aminopyridine and yohimbine. Am. J. Vet. Res. 44: 2037–2042.

FLETCHER, J. 1974. Hypersensitivity of an isolated population of red deer (*Cervus elaphus*) to xylazine. Vet. Rec. 94: 85–86.

FOLDES, F. F., Y. OHTA, Y. SHIWAKU, E. S. VIZI, J. J. VAN DIJK, AND K. MORITA. 1982. The influence of 4-aminopyridine on parasympathetic transmission. *In* Aminopyridines and Similarly Acting Drugs: Effects on Nerves, Muscles and Synapses, P. Lechat et al. (eds.). Pergamon Press, Toronto, Ontario, pp. 127–139.

FOLGERING, H., J. RUTTEN, AND S. AGOSTON. 1979. Stimulation of phrenic nerve activity by an acetylcholine releasing drug: 4-aminopyridine. Pflügers Arch. Eur. J. Physiol. 379: 181–185.

HARTHOORN, A. M. 1976. The Chemical Capture of Animals: A Guide to the Chemical Capture and Restraint of Wild and Captive Animals. Bailliére Tindall, London, 416 pp.

HATCH, R. C., N. H. BOOTH, J. D. CLARK, L. M. CRAWFORD, JR., J. V. KITZMAN, AND B. WALLNER. 1982. Antagonism of xylazine sedation in dogs by 4-aminopyridine and yohimbine. Am. J. Vet. Res. 43: 1005–1014.

HEBERT, D. M., AND R. J. MCFETRIDGE. 1981. Chemical Immobilization of North American Game Animals. Alberta Energy and Natural Resources, Fish and Wildlife Div., Edmonton, Alberta, 250 pp.

HSU, W. H. 1981. Xylazine-induced depression and its antagonism by alpha-adrenergic blocking agents. J. Pharmacol. Exp. Ther. 218: 188–192.

———, AND W. P. SHULAW. 1984. Effect of yohimbine on xylazine-induced immobilization in white-tailed deer. J. Am. Vet. Med. Assoc. 185: 1301–1303.

JACOBSEN, N. K. 1983. Effects of age and behavior of black-tailed deer on dosages of xylazine. J. Wildl. Manage. 47: 252–255.

JESSUP, D. A., W. CLARK, P. A. GULLET, AND K. R. JONES. 1983. Immobilization of mule deer with ketamine and xylazine, and reversal with yohimbine. J. Am. Vet. Med. Assoc. 183: 1339–1340.

———, ———, AND B. HUNTER. 1980. Animal Restraint Handbook. California Dept. Fish and Game, Sacramento, California, 183 pp.

KEEP, J. M. 1979. The sedation and immobilization of deer. *In* Proc. No. 49 Deer Refresher Course. The Australian Museum, Sydney, Australia, pp. 21–28.

KITZMAN, J. V., N. H. BOOTH, R. C. HATCH, AND B. WALLNER. 1982. Antagonism of xylazine sedation by 4-aminopyridine and yohimbine in cattle. Am. J. Vet. Res. 43: 2165–2169.

LÖFFELHOLZ, K., AND W. WEIDE. 1982. Aminopyridines and the release of acetylcholine. Trends Pharmacol. Sci. 3: 147–149.

LUCKENS, M. M., AND M. H. MALONE. 1973. Cardiovascular effects of reserpine-yohimbine mixtures on intact anesthetized dogs. J. Pharm. Sci. 62: 1286–1290.

MACKINTOSH, C. G., AND G. VAN REENEN. 1984. Comparison of yohimbine, 4-aminopyridine and doxapram antagonism of xylazine sedation in deer (*Cervus elaphus*). N.Z. Vet. J. 32: 181–184.

RENECKER, L. A., AND C. D. OLSEN. 1985. The use of yohimbine and 4-aminopyridine to antagonize xylazine in North American *Cervidae*. J. Am. Vet. Med. Assoc. 187: 1199–1201.

ROUGHTON, R. T. 1975. Xylazine as an immobilizing agent for captive white-tailed deer. J. Am. Vet. Med. Assoc. 167: 574–576.

SAGNER, G. F., F. HOFFMEISTER, AND G. KRONEBERG. 1968. Pharmacological basis of a new drug for analgesia, sedation and relaxation in veterinary medicine (Bay Va 1470). Dtsch. Tierärztl. Wochenschr. 75: 565–682.

SCHMIDL, J. A. 1974. Experimental use of ®Rompun on the exotic species. J. Zoo. Anim. Med. 5: 8–9.

STEELE, R. G. D., AND J. H. TORRIE. 1980. Principles and Procedures of Statistics. A Biometrical Approach, 2nd Ed. McGraw-Hill Book Co., Toronto, Ontario, 633 pp.

THORNE, E. T. 1980. Immobilization of wild ungulates: Considerations and drugs. Biotelem. Patient Monit. 7: 178–187.

———. 1982. Agents used in North American ruminant immobilization. *In* Chemical Immobilization of North American Wildlife, L. Nielsen et al. (eds.). Wisconsin Humane Soc., Inc., Milwaukee, Wisconsin, pp. 304–334.

WALLNER, B. M., R. C. HATCH, N. H. BOOTH, J. V. KITZMAN, J. D. CLARK, AND J. BROWN. 1982. Complete immobility produced in dogs by xylazine-atropine: Antagonism by 4-aminopyridine and yohimbine. Am. J. Vet. Res. 43: 2259–2265.