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Guanabenz in the horse – A preliminary report on clinical effects and comparison to clonidine and other alpha-2 adrenergic agonists

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Summary: In veterinary medicine, a number of alpha-2 receptor agonists are marketed as sedatives/hypnotics and analgesics, with their principal use being the chemical restraint of large and small animals. Guanabenz (Wytensin®) is an alpha-2 adrenergic receptor agonist marketed for use in humans as an anti-hypertensive agent. Recent reports indicate that guanabenz has been administered to horses in small doses (0.04 mg/kg) for its anti-hypertensive effects. While this offers both benefits of sedation of the horse as well as amelioration of pulmonary hypertension during running exercise and consequent Exercise-Induced Pulmonary Hemorrhage (EIPH), guanabenz is currently proscribed in most racing jurisdictions and its administration to a racing horse can lead to penalties. The *Association of Racing Commissioners International* (ARCI) lists guanabenz as an ARCI Class 3 agent; Class 3 agents include bronchodilators, anabolic steroids and other drugs with primary effects on the autonomic nervous system, procaine, antihistamines with sedative properties and diuretics and includes amitraz, clonidine, xylazine, detomidine, medetomidine, and romifidine. Guanabenz is unique among alpha-2 agonists in that it differentiates into E- and Z-forms (Fig. 1), with the Z-form lacking hypotensive properties, yet with both E- and Z-forms able to afford relief to cellular stresses related to inflammation or degenerative diseases. The objective of the study was a preliminary description of the pharmacological properties of guanabenz in comparison with clonidine and a number of other alpha-2 agonists. The goal was clinical evaluation of their sedative, analgesic and related activities with the goal of increasing our understanding of the clinical use of such medications and also as a possible prophylaxis for Exercise-Induced Pulmonary Hemorrhage. The clinical study of guanabenz and clonidine was performed in a complete crossover strategy using quantitative markers of sedation, antinociception, heart rate, blood and urine glucose following administration of each compound in five horses. Amitraz, detomidine, medetomidine, romifidine, and xylazine were studied in one horse each. The sedation was quantified by measuring head droop and locomotor activity, while antinociception was measured by Hoof Withdrawal Reflex Latency. Heart rates, urine glucose, urine production and urine specific gravities were also determined by standard clinical chemistry techniques. Guanabenz serum levels and related urinary guanabenz glucuronide levels were determined by established Liquid Chromatography-tandem Mass Spectrometric (LC-MS) methods. In result the clinically effective doses (0.2 mg/kg) of guanabenz produced a rapid and intense sedative effect, with sagging of the lower lip, sunken eyelids, and marked head droop corresponding to plasma guanabenz concentrations that peaked at 120 ng/mL at 2.5 min post-injection (Fig. 2). The initial head height above the ground is considered 100 %, and head heights fell to values ranging 18–40 % with guanabenz, all of which are greater than a 50 % reduction in head height, considered a full clinically useful sedative effect. Despite the intensity of the sedation, all horses remained standing and were able to walk, and the sedation and head droop responses were rapidly reversed by intravenous administration of the alpha-2 receptor antagonist yohimbine, reversals occurring within 10 min of administration. As a pilot investigation this study was extended to six other members of the alpha-2 agonist group, clonidine administered to five horses, and amitraz, detomidine, medetomidine, romifidine, and xylazine to one horse each. Hoof Withdrawal Reflex Latency evaluation demonstrated the considerable analgesic properties of guanabenz, greater than the corresponding potencies among clonidine, detomidine, romifidine, medetomidine and xylazine. Heart rate monitoring showed guanabenz as possessing capacity for prolonged bradycardia, with effects of a single dose lasting for up to 3.5 hr, in contrast with clonidine (1 hr), amitraz (2 hr), detomidine (<1 hr), medetomidine (1 hr), romifidine (2 hr), and xylazine (<1 hr). Peak urine production following guanabenz administration occurred between 1.5 and 3.0 hr after administration (Fig. 6), as indicated by the steeper decline of the urine volume curve during that period. Urine specific gravity dropped to a low of about 1.006 at 2.0 hr after administration and remained at this level for ~1.0 hr. Urine pH remained at 8, and urine protein was negative throughout testing. The other alpha-2 agonists evaluated also caused an increased urine production with a concomitant decrease in specific gravity. The effect of guanabenz had the longest duration on increased urine volume, lasting about 3.0 hr. Xylazine had the shortest diuretic effect, persisting for only about 1.0 hr. Guanabenz along with romifidine and detomidine induced glucosuria whereas other alpha-2 agonists did not. Hyperglycemia and the corresponding glucosuria resulted in a significant diuresis, as shown by the cumulative urine volume. Guanabenz along with amitraz, detomidine and xylazine also produced measurable sedation presenting as reduced locomotor activity (Table 1). While all alpha-2 agonists showed qualitatively similar pharmacological responses, only guanabenz produced an intense and relatively prolonged antinociceptive response. The study is limited by the number of horses examined (five each for guanabenz and clonidine, five for repeat studies that included yohimbine antagonism, and one each for the other agonists). Study design was focused on clinical evaluation of agonist similarities and differences and thus did not specifically generate data for detailed statistical evaluation. In conclusion these studies show that a 100 mg IV dose of guanabenz rapidly induces clinically useful sedation, analgesia and antinociception effects that are more intense and considerably longer-lasting than those produced by other alpha-2 receptor agonists evaluated. Guanabenz also remains detectable in serum up to 8-hours following administration at doses as low as 0.04 mg/kg. In the work reported here, guanabenz administered at 0.2 mg/kg IV showed peak concentrations in serum of 120 ng/mL at 2.5 min and was detectable for up to 4 hr with its glucuronide metabolite peaking at 120 min post-administration. Although we did not investigate the combination of guanabenz with opioid drugs such as butorphanol for pain management, guanabenz may well be a drug of choice among the other alpha-2 agonists evaluated in this study for administration with opioids for pain management based on maintaining maximum levels of analgesia for longer periods of time. These experiments suggest considerable clinical potential for guanabenz as a sedative and a relatively long-lasting analgesic in equine medicine. Based on these pharmacological properties, guanabenz and related alpha-2 agonists also have considerable potential for clinical use in equine medicine.

Keywords: guanabenz, alpha-2 adrenergic agonists, sedation, antinociception, exercise-induced pulmonary hypertension

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Introduction

Guanabenz (2- (2,6-dichlorobenzylidene)hydrazinecarboximidamide, $C_8H_8Cl_2N_4$, molar mass 231.08 g·mol^{-1}) belongs to the class of drugs known as dichlorobenzenes which includes certain nonsteroidal anti-inflammatory agents such as diclofenac and long acting beta-2 adrenergic receptor agonists among others (*Francis* 1976, *Procopiou* et al. 2010, *Akgul* et al. 2018). Guanabenz, as an alpha agonist with high affinity for the alpha-2 adrenergic receptor, is used in human medicine as an antihypertensive drug (*Baum* and *Shropshire* 1976, *Wiley* 2007). Guanabenz is similar to clonidine in that the two compounds share the 2,6-dichlorophenyl ring structure (*Giovannitti* et al. 2015). Alpha-2 receptor agonists display an array of affinities for alpha-2 receptors over alpha-1, such that clonidine has an affinity predilection of 200:1 whereas a compound like dexmedetomidine is much more exclusive with a 1620:1 ratio (*Giovannitti* et al. 2015). Guanabenz is closer to clonidine in its alpha-2/alpha-1 affinity (*Maze* and *Tranquilli* 1991). Owing to its sedative and analgesic properties, guanabenz has gained significant interest among horse owners as a calming agent (*Colahan* et al. 1996), whether injected by a veterinarian for therapeutic purposes or by owners or trainers desiring increased horse control. Interest in guanabenz exists since most of the behavior-altering drugs used today in the horse industry originated from harsher psychotropic drugs used to treat human mental illness, such as antipsychotic drugs acepromazine, chlorpromazine, fluphenazine and reserpine (*Tobin* 1981). Note that the latter group of compounds were originally introduced in the 1950s as tranquilizers in human medicine but went on to be primarily used for treatment of psychotic individuals (*Tobin* 1981); the term tranquilizer is still rather loosely used in equine circles to describe any drug that allows a horse to remain conscious and standing but enables ready treatment by a veterinarian (*Tobin* 1981). Guanabenz would represent a welcome substitute sedative. However, note that the US Equestrian Federation will not accept any medication reports for competing horses that list use of guanabenz (https://www.usef.org/forms-pubs/2Zp2C_YKs4s/2022-equine-drugs-medications).

Guanabenz may be an antihypertensive used to control blood pressure, but it is thought to produce a rapid acting, long lasting and safe sedative and analgesic effect that is selectively reversible in a subject animal (*Tobin* 2001). The antihypertensive effect is thought to be due to central alpha-adrenergic stimulation, which results in a decreased sympathetic outflow to the heart, kidneys, and peripheral vasculature in addition to a decreased systolic and diastolic blood pressure and a slight slowing of pulse rate. In addition, there are extra-junctional alpha2-adrenoceptors, e.g. in the vascular walls, and their activation causes vasoconstriction. Another consideration is that alpha-2 agonists typically cause presynaptic inhibition of the alpha2 receptor which suppresses norepinephrine release and suppression of sympathetic outflow (*Maze* and *Tranquilli* 1991). Chronic administration of guanabenz also causes a decrease in peripheral vascular resistance and the primary target is considered to be alpha-2 adrenergic receptors (*Baum* and *Shropshire* 1976, *Colahan* et al. 1996), although imidazoline receptors have also been suggested as targets (*Bousquet* and *Feldman* 1999). Understanding the pharmacodynamic effects of guanabenz would provide a valuable asset to horse owners, trainers and veterinarians, particularly owing to its increasing use in racing and other performance horses as a calming agent, given primarily to take the edge off excitable and overly anxious horses and to improve their focus and manageability (*O'Connor* 2015).

Fig. 1 shows the structure of commercially available guanabenz acetate which releases acetic acid on exposure to body fluids to release the major E-azine form of guanabenz. *Kathuria* et al. (2019) recently reported the minor configurational Z-azine isomer which is less stable by 2.13 kcal/mole and therefore present in lower concentrations on the order of 5% in crystal form. *Xie* et al. (2021) recently reported that the Z-form lacks hypotensive properties, yet both E- and Z-forms afford relief to stresses of the endoplasmic reticulum related to inflammation or degenerative diseases.

The alpha-2 receptor agonists, of which clonidine, xylazine and detomidine are the best-known examples, were first synthesized in the early 1960s and found to produce vasoconstriction when applied topically (*Stähle* 2001). Clinical testing of clonidine as a nasal decongestant revealed that it caused hypotension, sedation, and bradycardia in man, which led to its introduction as an antihypertensive (*Stähle* 2001). The alpha-2 agonists reduced arterial blood pressure by mediating both cardiac output and peripheral resistance (*Hoffman* 2001, *Hoffman* and *Taylor* 2001).

One rationale for guanabenz administration to racehorses is that most experience pulmonary hypertension during running exercise, leading to exercise-induced pulmonary hemorrhage (EIPH), a considerable problem in the horse racing industry (*Hinchcliff* et al. 2015). EIPH acutely interferes with the racing performance of horses by compromising the exchange of O_2 and CO_2 in the alveolar capillaries, and repeated bouts of EIPH result in chronic and cumulative damage to the lung (*O'Callaghan* et al. 1987). For these reasons, horsemen have

long sought effective prophylactic approaches for the alleviation or prevention of EIPH. Among the possible prophylactic approaches to EIPH, equine veterinarians and horsemen reportedly have administered ground-up guanabenz tablets resuspended in an alcohol solution (*Taylor* et al. 2000). This formulation is administered in small (10–20 mg) doses shortly before post time to reduce the intensity of the racing-related pulmonary hypertension and, by extension, the associated EIPH.

However, guanabenz is listed by the *Association of Racing Commissioners International* (ARCI) as an ARCI Class 3 agent (ARCI 2020). As such, guanabenz is considered to have significant potential to influence the outcome of a race, and its administration to a horse shortly before post time clearly contravenes the rules of racing in most jurisdictions. It has therefore been the target of multi-analyte testing methods designed for plasma, urine and even hair analyses (*Wong* et al. 2018, 2020a, 2020b). The objective of this study was an initial determination of the pharmacological effects of guanabenz in comparison to clonidine and a preliminary assessment relative to other alpha-2 agonists including xylazine, detomidine, medetomidine, romifidine, and amitraz. Pharmacological effects measured included head droop, antinociception, heart rate, and urine output.

Material and methods

Horses

Five mature Thoroughbred mares weighing 448 to 489 kg from the University of Kentucky pool of horses were used for this study in comparison of guanabenz and clonidine. Mares were chosen as they are preferred for urine collection since they can be readily catheterized. The animals were maintained on grass hay and feed (12% protein), which was a 50:50 mixture of oats and an alfalfa-based protein pellet. Horses were fed twice a day. The animals were vaccinated annually for tetanus and were dewormed quarterly with ivermectin (MSD Agvet, Rahway, NJ). A routine clinical examination was performed before each experiment by an accredited equine veterinarian to assure that the animals were healthy and sound. Horses were returned to the UK herd between experiments. Because of the critical role of superficial skin temperature in these experiments, no heat lamp experiments were performed when the ambient temperature was less than 10 °C, and experiments were therefore organized in accordance with daily weather reports. Animals used in these experiments were managed according to the rules and regulations of the University of Kentucky Institutional Animal Care Use Committee (IACUC), which also approved the experimental protocol.

Drug sources

Drugs were purchased from the following vendors: Guanabenz acetate (Research Biochemicals Corp., Natick, MA, now part of Sigma-Aldrich, St. Louis MO); Amitraz (Riedel-de Haën, Seelze, Germany); Detomidine-HCl as Dormosedan (Pfizer Animal Health, West Chester, PA); Xylazine (Cutter Laboratories, Topeka, KS); Medetomidine as Domitor (Orion Pharma, Espoo, Finland); Romifidine-HCl as Sedivet (Boehringer-Ingelheim, Ridgefield, CT); Clonidine (Sigma-Aldrich); and Yohimbine (Sigma-Aldrich).

Drug dosing protocol

Pharmacological effects were determined following intravenous administration of 0.2 mg/kg of guanabenz acetate (approximately equivalent to 100 mg guanabenz to a 1200 lb. horse) dissolved in 3 mL ethanol. Note that 3 mL ethanol introduced into the 54 L blood in an average 500 kg horse results in only 0.005% ethanol, far below the 0.08% level considered legal intoxication in man (*Chapman* and *Rudrum* 1978). Medications were administered slowly (about one or two milliliters per five seconds) into the jugular vein. Head droop, hoof withdrawal reflex latency, locomotor activity, heart rate, urine glucose, urine production and urine specific gravity were measured. These results were compared to similar effects measured following administration of full doses (*Valverde* 2010) of other alpha-2 agonists, including: amitraz (0.015 mg/kg), clonidine (0.02 mg/kg), detomidine (0.04 mg/kg), medetomidine (0.01 mg/kg), romifidine (0.1 mg/kg), and xylazine (1 mg/kg). Although guanabenz and clonidine were administered to five horses each, the comparative doses of other agents were administered to one horse as an initial evaluation of clinical effectiveness, selecting randomly from among the five horses for treatments with the other five agents. Dosages of each drug and the corresponding number of horses are summarized in Table 1. At least one week elapsed between all treatments. The order of dosing was randomized.

Measurement of guanabenz and its glucuronide in serum

Methods for guanabenz IV dosing and measurement by liquid chromatography-tandem mass spectrometry (LC/MS/

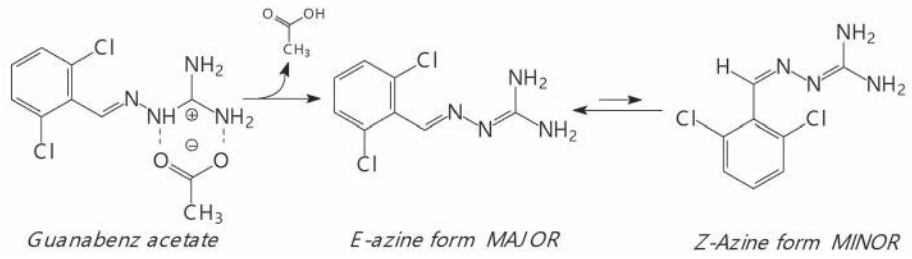

Fig. 1 The guanabenz acetate complex releases acetic acid on exposure to acid in body fluids, forming the predominant E-azine configurational isomer of guanabenz. This compound is in equilibrium with the minor rearrangement Z-azine structure. Adapted from *Kathuria* et al. (2019). | *Der Guanabenzacetat-Komplex setzt bei Kontakt mit Säure in Körperflüssigkeiten Essigsäure frei und bildet das vorherrschende E-Azin-Konfigurationsisomer von Guanabenz. Diese Verbindung befindet sich im Gleichgewicht mit der geringfügigen Umlagerung der Z-Azin-Struktur. Angepasst von Kathuria et al. (2019).*

MS) have been described in detail (*Harkins* et al. 2003). Serum samples (10 ml) were collected by direct venipuncture from the jugular vein into serum collection tubes (Vacutainer Systems, Becton Dickinson, Franklin Lakes, NJ). Serum was separated and frozen in 5 aliquots at -20 °C until assayed. Mass spectral data were acquired on extracts on a Quattro-II MS/MS (Micromass, Wilmslow, UK) operated in ESI positive mode for acquisition of the guanabenz *m*/z 231→72 transition and its ratio to clenbuterol *m*/z 277→203 transition as internal standard. During the first 8 h after administration, complete urine collection was accomplished with a Foley catheter (24 Fr, Rusch Inc, Duluth, GA, 30136) and an attached plastic bag. At 24 h after administration, a Harris flush tube (24 Fr × 60 in; Seamless, Ocala, FL) was used to collect a maximum of 300 ml urine. Urine was placed in 5 aliquots of 100 ml each and stored at -20 °C until assayed. Guanabenz glucuronide was quantified in urine following a 100 mg IV dosed horse as follows: 1.0 mL of urine was diluted with an equal volume of acetonitrile (HPLC grade, Fisher Chemicals, Fair Lawn, NJ), centrifuged briefly, and the supernatant recovered and mixed 1:1 with acetonitrile: 0.05 % formic acid (aq), 50:50. This mixture was infused at 1.2 mL/hr via a Harvard syringe pump into the electrospray probe of the Quattro II tandem mass spectrometer set in positive ion mode for acquisition of the *m*/z 247 fragment signifying the presence of the glucuronide. All spectra were optimized by combination of 1 to 2 minutes of uniformly acquired data, background subtraction, and peak smoothing. Also acquired were individual *m*/z values between 423 and 428 for confirmation of the guanabenz glucuronide presence by comparison to published relative responses (*Harkins* et al. 2001, 2003).

Urine chemistry

Urine glucose, urine production and specific gravity were monitored following IV injection of the alpha-2 agonists. Urine was collected using a Foley catheter for 8 hr after administration. The catheter was drained into a volumetric flask to measure urine volume. Urine specific gravity was measured with a refractometer (URN-NE, Atago, Japan), glucose was measured with DiaScreen 3 urine sticks (ChroniMed, Minneapolis, MN) and pH was measured with a Fisher Accumet model 230 pH meter.

Quantitation of pharmacological effects: Head droop

Sedation was assessed by measuring the degree of head droop following administration of each alpha-2 agonist. This was performed in five horses administered guanabenz and clonidine, but only in a single horse with detomidine, romifidine, medetomidine, and xylazine. Horses were chosen at random for treatment following minimum 7 day withdrawal periods. A pre-treatment height (from floor to chin) was determined at -10, -5 and 0 min before intravenous injection of the alpha-2 agonists. The degree of head droop was then measured at 5, 10, 15, 20, 25, and 30 minutes after injection and every 15 minutes thereafter until head droop measurements returned to baseline values. All related clinical signs associated with the sedative effects in horses, such as ataxia and drooping of the eyelids and lower lip, were also reported. The clinician who administered the medication measured the head height by directly observing the animals in very quiet environment using measurement tape attached to the wall where horse was standing. Clinicians were not blinded to the treatment group. In a

Table 1 Summary of alpha-2 agonist activities including dosage applied to study head droop, antinociception, decreased heart rate and glucosuria. Also listed are examples of sedation effects and the dose used in a 500 kg horse to generate sedation. Experiments with guanabenz and clonidine were performed with five horses, and average results are shown. Experiments with detomidine, romifidine, medetomidine, xylazine and amitraz were performed each in a single horse. Normal (i.e., no effect) responses would be 1) head droop duration, 0 hr; 2) antinociception effect, 100 % of control; 3) antinociception duration, 0 hr; 4) glucosuria, absence (-); 5) sedation as reduced locomotor activity, 0 min. | *Zusammenfassung der Wirkungen von Alpha-2-Agonisten, einschließlich der Dosierung, die zur Untersuchung von Kopfschlappe, Antinozizeption, verringerter Herzfrequenz und Glucosurie verwendet wurde. Aufgeführt sind auch Beispiele für Sedierungseffekte und die Dosis, die bei einem 500 kg schweren Pferd verwendet wurde, um eine Sedierung zu erzeugen. Experimente mit Guanabenz und Clonidin wurden mit fünf Pferden durchgeführt, und es sind die durchschnittlichen Ergebnisse angegeben. Experimente mit Detomidin, Romifidin, Medetomidin, Xylazin und Amitraz wurden jeweils an einem einzigen Pferd durchgeführt. Normale (d. h. keine Wirkung) Reaktionen wären: 1) Dauer des Kopfhängens, 0 Stunden; 2) Antinozizeptionseffekt, 100 % der Kontrolle; 3) Dauer der Antinozizeption, 0 Stunden; 4) Glucosurie, Abwesenheit (-); 5) Sedierung als verringerte Bewegungsaktivität, 0 Minuten.*

Compound	Dosage	Head droop duration	Antinociception effect	Antinociception duration	Decreased HR duration	Glucosuria	Sedation as reduced locomotor activity	Sedation dose	Number of horses examined
	mg/kg	hr	% of control	hr	hr	presence (+) or absence (-)	min	mg IV	
Guanabenz	0.2	3.5	300 %	3	3.5	+	240	100	5
Clonidine	0.02	1.5	150 %	0.75	1.5	-	--	--	5
Detomidine	0.04	1.5	150 %	1.5	1.5	+	120	10	1
Romifidine	0.1	≤ 1.0	300 %	0.25	1.5	+	--	--	1
Medetomidine	0.01	≤ 1.0	300 %	0.5	1	-	--	--	1
Xylazine	1	≤ 1.0	300 %	0.25	0.75	-	15	167	1
Amitraz	0.015	≤ 1.0	--	--	1.5	-	120	25	1

separate experiment to evaluate the reversal effect of an alpha-2 receptor antagonist, the same protocol was followed as described above, but 30 min following injection of guanabenz or clonidine, the horses received an IV injection of 0.12 mg/kg yohimbine powder dissolved in 2 mL of dimethyl sulfoxide (DMSO) and head droop was measured roughly every 5 min. This was performed in five horses administered guanabenz and clonidine only. Horses were chosen at random for treatment following minimum 7 day withdrawal periods.

Measurement of pharmacological effects: Analgesia

Thermal antinociception, which has been used as a measure of analgesia, was determined with a heat projection lamp as described previously (*Harkins* et al. 1996). Briefly, the hair on the dorsal and lateral sides of the foreleg pasterns was clipped and the pastern was blackened with stamp pad ink to insure equal and consistent heat absorption for all horses. Focused radiant light/heat was used as a noxious stimulus and was directed onto the pastern of the horse, from a constant distance of 30 cm, to elicit the classic flexion-withdrawal reflex. Hoof withdrawal reflex latency (HWRL) was defined as the time between illumination and withdrawal of the hoof. The reflex times were adjusted by varying the intensity of the heat output with a rheostat so that the HWRL for control measurements was 3–4 sec, with the actual HWRL recorded on an electronic timer built into the lamp. The duration of light exposure to the pasterns was limited to 10 sec to prevent skin damage. A secondary unfocused light beam (sham light) was used to confound the horse, reducing the possibility that the flexion-withdrawal reflex was to visual rather than thermal perception of the focused light beam (*Harkins* et al. 1996).

HWRL was measured at -30 and -15 min and immediately before injection, and these times (-30, -15, and 0 min) were used to establish a baseline value for HWRL in each horse. The HWRL was then measured at 5 and 15 min after injection and every 15 min thereafter until the HWRL returned to control values. The HWRL was expressed as a percent of baseline values (100%), with 9 seconds corresponding to maximum analgesic effect (300%).

Measurement of pharmacological effects: heart rate

Heart rates (HR) were recorded at 1 min intervals during each experiment by an on-board heart rate computer (Polar CIC, Inc., Port Washington, NY). An elastic strap with a receiver and transmitter attached was placed around the chest of the horse. The transmitter was connected to two electrodes placed on the shaved areas of the sternum and left side of the anterior chest. Electrode gel was used to insure proper conduction of the HR signal. Each animal served as its own control meaning that heart rate was measured in the same animal with and without administration of drugs. Control animals were injected with ethanol-based solutions containing no drugs.

Locomotor response quantitation

Methods for locomotor response quantification are as previously described (*Harkins* et al. 1997). In brief, locomotor activity of the horse was detected individually by four Minibeam sensors spaced equally around a 3.5×3.5 m stall and recessed into the walls 45 cm above the dirt floor. Each time the horse disrupted the beam of light a count was scored. The output from the four sensors was summed and recorded on a data logger. Results are reported as the length of time observed as relative inactivity.

Results

Guanabenz levels

Horses dosed with 0.2 mg/kg guanabenz showed peak blood concentrations averaging 120 ng/ml by 2.5 min post-administration (Fig. 2). The principal glucuronide metabolite then peaked in urine at 120 min post-injection (Fig. 2).

Sedation

In the basic walking pace, a horse should be able to go forward calmly but freely and energetically, stretching his neck and consequently raising his back. Although horses remained standing and were able to walk following IV guanabenz administration (0.2 mg/kg), the agent produced rapid, profound sedation as evinced by relaxation of the lower lip, sunken eyelids, and extreme head droop (Fig. 3). As suggested by the rapid onset of the head droop response, the horses were clinically depressed within minutes of the guanabenz administration, and the profound head droop persisted for 3.5 hr after drug administration (Fig. 3a). This figure also illustrates the rapid reversal following yohimbine injection; 2 min following IV injection of yohimbine (0.12 mg/kg), head height was ~50% of pre-treatment value, and by 5 min after injection, head height was within normal limits and the horse was clinically alert. Clonidine and detomidine also produced profound head droop for 1–1.5 hr, with the other alpha-2 agonists evaluated all producing head droop of shorter duration. Head height above the ground (HHAG) fell from baseline 100% to following approximate percentage values for each drug: guanabenz, 40%; clonidine, 32%; detomidine, 23%; romidine, 20%; medetomidine, 18%; xylazine, 25%, and we note that *Rodrigues de Oliveira* et al. (2021) rated reductions of the HHAG by 50% or more as sufficient, i.e., complete sedation.

Antinociception

Figure 4 illustrates the rapid onset of antinociception as determined by Hoof Withdrawal Reflex Latency following administration of several of the alpha-2 agonists. Intravenous administration of 0.2 mg/kg of guanabenz provided maximal measurable antinociception (300% of control value) by 10 min after injection, which persisted through the 0.75 hr testing point. The antinociceptive response had not returned to control values until 6 hr post-administration, by far the longest antinociceptive response to any of the alpha-2 agonists evaluated (Fig. 4a). Romifidine, medetomidine, and xylazine provided maximal antinociception for 0.25, 0.5, and 0.25 hr, respectively (Fig. 4d-f). Clonidine and detomidine failed to provide maximal antinoci-

ception, although their partial antinociceptive responses persisted for ~0.75 and 1.5 hr, respectively (Fig.s 4b-c).

Heart rate

Fig. 5 shows the decreased HR following injection of guanabenz, which persisted for ~3.5 hr. Amitraz and romifidine decreased HR for ~1.5 hr. Clonidine, detomidine, medetomidine, and xylazine decreased HR for ~1.5, 1.5, 1.0, and 0.75 hr, respectively.

Urine chemistry

Guanabenz administration produced a significant hyperglycemia and glucosuria (normal blood and urine glucose ranges are 70–140 and 0 mg/dL, respectively (*Rose* and *Hodgson* 1994)). Fig. 6 shows immediate measurable effects within 8 min post-injection. At ~2.0 hr after administration, glucose started to appear in the urine (Fig. 7a). Urine glucose continued to rise late in the experiment, even though blood glucose returned to normal values by 8 hr after administration. Amitraz, romifidine, and detomidine also produced glucosuria (Fig. 7).

Peak urine production occurred between 1.5 and 3.0 hr after guanabenz administration, as indicated by the steeper slope of the urine volume curve during that period. Thereafter, urine production was minimal, essentially returned to baseline (pre-injection) levels (Fig. 6). Similarly, urine specific gravity dropped to a low of about 1.006 at 2.0 hr after administration, remained at this level for ~1.0 hr, and gradually returned to control value, with the lowest urinary specific gravity values corresponding with peak urine production (Fig. 6). Urine pH remained at 8, and urine protein was negative throughout the testing (data not shown).

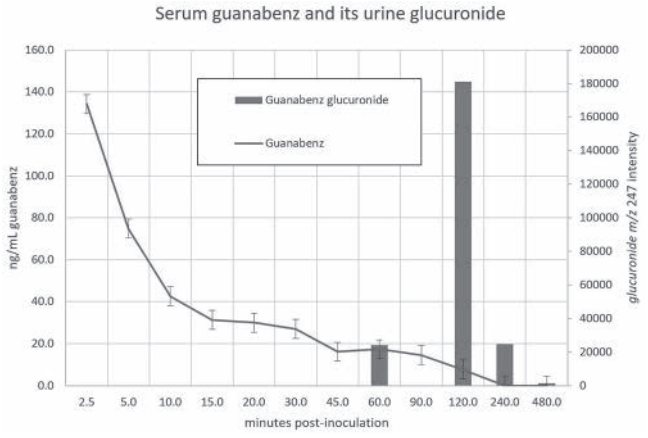

Fig. 2 Serum guanabenz concentrations following IV injection of 0.2 mg/kg guanabenz (average of five horses). Also shown is the appearance of the glucuronide metabolite in one of the horses peaking at 2 hr post-injection as measured in urine by the compound's m/z 247 intensity. | *Guanabenz-Konzentrationen im Serum nach intravenöser Injektion von 0,2 mg/kg Guanabenz (Durchschnitt von fünf Pferden). Dargestellt ist auch das Auftreten des Glucuronid-Metaboliten bei einem der Pferde, das 2 Stunden nach der Injektion seinen Höhepunkt erreicht, gemessen im Urin anhand der m/z 247-Intensität der Verbindung.*

The other alpha-2 agonists also caused an increased urine production with a concomitant decrease in specific gravity. The effect of guanabenz (Fig. 7a) had the longest duration on increased urine volume, lasting about 3.0 hr. Xylazine (Fig. 7f) had the shortest polyuric effect, persisting for about 1.0 hr.

Discussion

The incidence and prevalence of exercise-induced pulmonary hypertension in racehorses has prompted horse owners to explore the use of guanabenz and related alpha-2-agonists as prophylactic treatments due to their anti-hypertensive properties, despite sanctions against such activities by local racing rules and the ARCI listing of guanabenz as a Class 3 agent. However, as pointed out by *Valverde* (2010) and *Ringer* et al. (2013), side effects of such agents may actually include transient hypertension. Adverse effects can include decreased cardiac output, respiratory depression, increased vagal tone and systemic vascular resistance, and bradycardia. The bradycardia is in response to transient initial hypertension due to vasoconstriction amounting to a compensatory reaction. This occurs in biphasic pattern, with hypotension re-

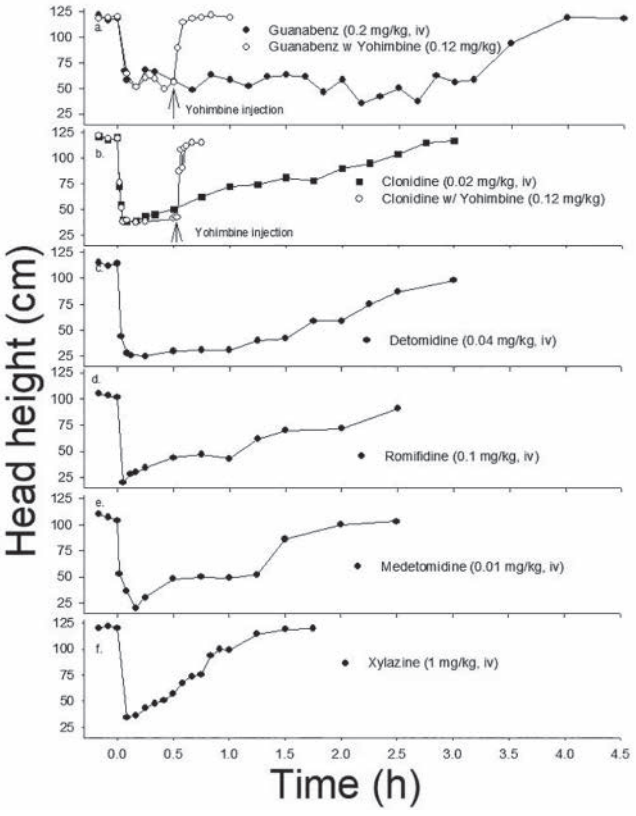

Fig. 3 Head droop following IV injection of the alpha-2 agonist agents. The sedative effects of a) guanabenz and b) clonidine were rapidly reversed by yohimbine. Experiments with guanabenz, clonidine and yohimbine were performed with five horses, and average results are shown. Experiments with detomidine, romifidine, medetomidine and xylazine were performed each in a single horse. | *Absenkung des Kopfes nach intravenöser Injektion der Alpha-2-Agonisten. Die sedierende Wirkung von a) Guanabenz und b) Clonidin wurde durch Yohimbin rasch aufgehoben. Die Versuche mit Guanabenz, Clonidin und Yohimbin wurden mit fünf Pferden durchgeführt, und es werden durchschnittliche Ergebnisse gezeigt. Experimente mit Detomidin, Romifidin, Medetomidin und Xylazin wurden jeweils an einem einzigen Pferd durchgeführt.*

sulting after the initial vasoconstrictive effects subside (*Nann* 2010). This is in essence a homeostatic mechanism called the "Baroreceptor Reflex" in which baroreceptors increase vagal nerve firing to induce a decrease in heart rate. Note that alpha-2 agonist administration suppresses sympathetic outflow and the reverse autonomic response – a compensatory rise in heart rate – does not occur (*Niederhoffer* et al. 2004). Despite the complexities of guanabenz's anti-hypertensive effects, we investigated its sedative and analgesic effects as beneficial pharmacological effects in horses. Note that, as reported previously (*Harkins* et al. 2003), guanabenz remained detectable in serum up to 8 hours following administration at doses as low as 0.04 mg/kg dose. Note that in the work reported here guanabenz inoculated at 0.2 mg/kg peaked in serum at 120 ng/mL at 2.5 min and was visible to 4 hr with the appearance of a glucuronide peaking at 120 min post-injection. Given a relatively short half-life of 56 min for guanabenz inoculated at 0.2 mg/kg (*Harkins* et al. 2003), after 4 half-lives – 3.7 hr – the amount of drug would be considered negligible regarding its therapeutic effects. One may surmise possible activity of the glucuronide or additional metabolites in extending guanabenz pharmacological activity to 300 min, a possibility described by *Yang* et al. (2017), who claim the majority of drug glucuronides are pharmacologically inactive; however, in certain incidences glucuronides have been shown to be equally or more effective than the parent drug. A summary of findings follows:

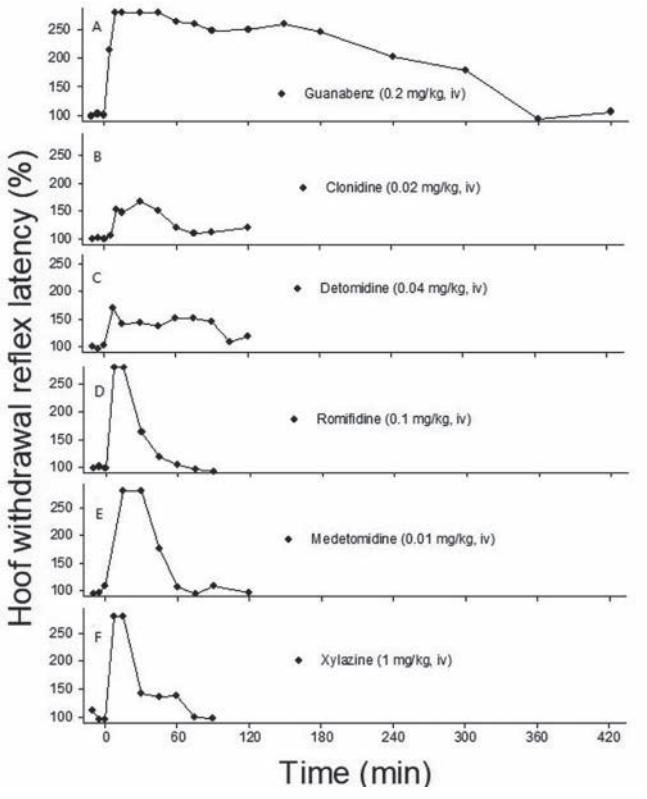

Fig. 4 Hoof Withdrawal Reflex Latency (HWRL) following IV injection of the alpha-2 agonists. Experiments with guanabenz and clonidine were performed with five horses, and average results are shown. Experiments with detomidine, romifidine, medetomidine and xylazine were performed each in a single horse. | *Latenzzeit des Hufrückzugsreflexes (HWRL) nach IV-Injektion der Alpha-2-Agonisten. Die Versuche mit Guanabenz und Clonidin wurden mit fünf Pferden durchgeführt, und es werden die durchschnittlichen Ergebnisse gezeigt. Versuche mit Detomidin, Romifidin, Medetomidin und Xylazin wurden jeweils an einem einzigen Pferd durchgeführt.*

Sedation

The sedative effects of the alpha-2 agonists were similar and included lowering of the head, sinking of the eyelids and lower lip, and ataxia. If the initial head height above the ground is considered 100%, head heights fell to values ranging 18–40%, all of which are considered less than a 50% cut-off for complete sedation (*Rodrigues de Oliveira* et al. 2021). Although the horses were not evaluated by the EquiSed approach of *Rodrigues de Oliveira* et al. (2021), we note that the observation of ataxia corresponds to their highest scale value for postural instability, including intense swaying and risk of falling down.

Different studies have used various criteria to measure sedation (*Tronicke* and *Vocke* 1970, *Jochle* and *Hamm* 1986, *England* et al. 1992, *Bryant* et al. 1999). The sedative effects of many of the alpha-2 agonists have been quantified with an equine

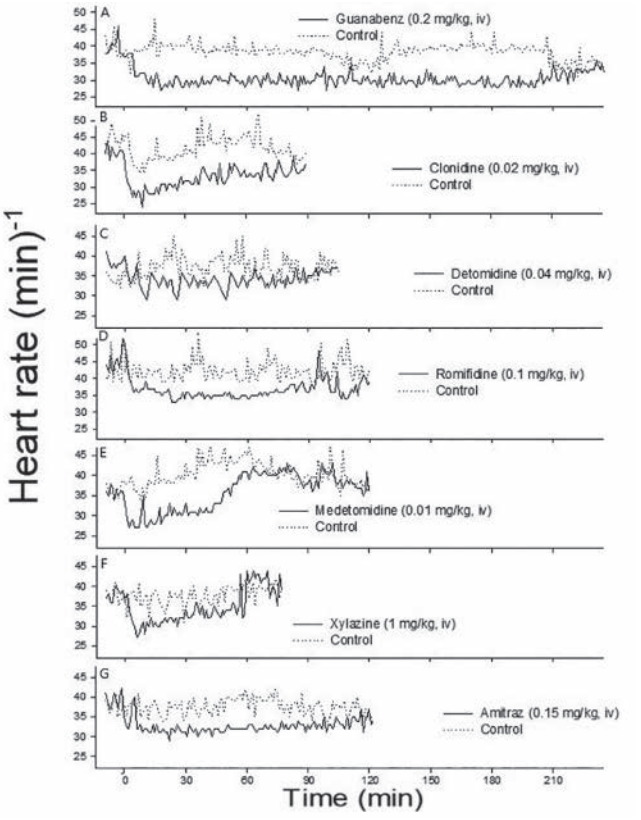

Fig. 5 Heart rates following IV administration of the alpha-2 agonists, showing experimental as solid line and control as dotted line responses. Experiments with guanabenz and clonidine were performed with five horses, and average results are shown. Experiments with amitraz, detomidine, romifidine, medetomidine and xylazine were performed each in a single horse. | *Herzfrequenzen nach intravenöser Verabreichung der Alpha-2-Agonisten, wobei die experimentellen Reaktionen als durchgezogene Linie und die Kontrollwerte als gestrichelte Linie dargestellt sind. Die Experimente mit Guanabenz und Clonidin wurden mit fünf Pferden durchgeführt, und es werden die durchschnittlichen Ergebnisse gezeigt. Die Versuche mit Amitraz, Detomidin, Romifidin, Medetomidin und Xylazin wurden jeweils an einem einzigen Pferd durchgeführt.*

locomotor chamber (*Harkins* et al. 1997). Amitraz (25 mg, IV; 0.05 mg/kg) significantly reduced locomotor activity for about 120 min post-administration. Larger doses (50 and 75 mg, IV; 0.1 and 0.15 mg/kg) reduced spontaneous activity for 180 and 240 min, respectively. Detomidine (10 and 20 mg, IV; 0.02 and 0.04 mg/kg) reduced locomotor activity for about 120 and 240 min, respectively, and xylazine (167 and 500 mg, IV; 0.33 and 1.0 mg/kg) reduced locomotor activity for about 15 and 30 min, respectively. Guanabenz (100 mg, IV; 0.2 mg/kg) significantly reduced locomotor activity for about 240 min post-administration (*Harkins* et al. 2003).

Daunt and *Steffey* (2002) reviewed alpha-2 adrenergic agonists effects in horses and noted head droop among them. For example, *Lawless* et al. (2021) studied head height as a means of gauging comparative sedative effects of detomidine and romifidine during laparoscopic ovariectomy. In a similar study that measured head droop (*Queiroz-Neto* et al. 1998), amitraz produced a dose-dependent response. The lowest dose (0.05 mg/kg) produced significant head droop from 5–60 min post-administration, and the highest doses administered (0.15 mg/kg) produced significant head droop from 5–150 min after administration. Guanabenz at 0.2 mg/kg exceeded the head droop effect of the highest amitraz dosage by about 90 min (to 240 min), although this could be rapidly reversed by yohimbine administration within about 10 min. The findings of strong sedation are consistent with those reported by *Colahan* et al. (1996) who also found decreased heart rate in exercising Thoroughbreds treated with 0.08 mg/kg guanabenz.

Alpha-2 agonists are used in Veterinary Medicine in order to induce dose-dependent sedation, analgesia, and skeletal muscle relaxations, and they are used as sedatives and analgesics to facilitate handling, clinical examinations, clinical procedures, and minor surgical procedures, and for use as a preanesthetic prior to the induction of general anesthesia. The longer duration of analgesia and sedation induced by guanabenz might be more attractive for some clinicians for lengthy clinical examinations and minor surgical procedures. Although we did not investigate the combination of guanabenz with opioid drugs such as butorphanol for pain management, it is possible that guanabenz might be the drug of choice among the other al-

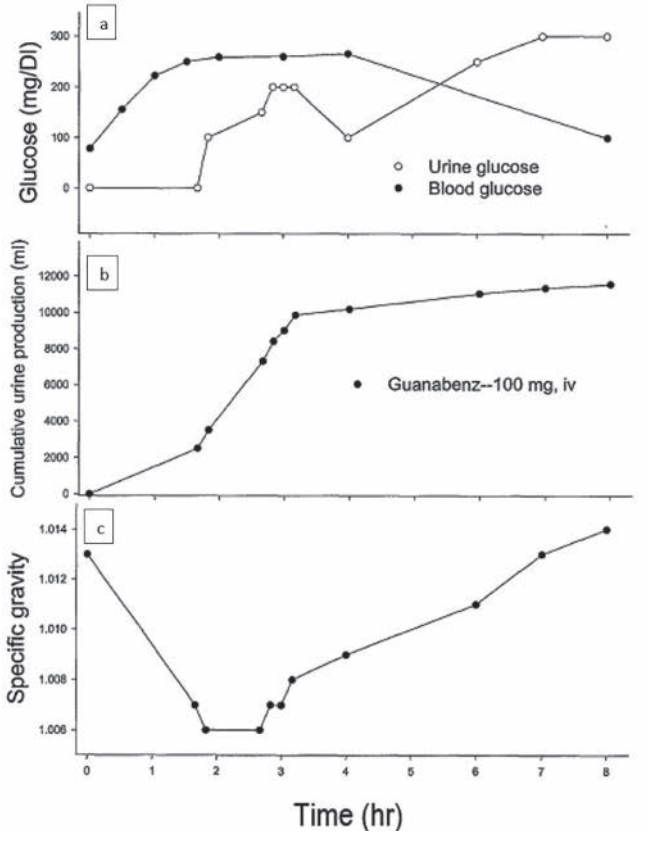

Fig. 6 Guanabenz effects on blood and urine chemistry during the 8 min period following 0.2 mg/kg injection. A) blood and urine glucose levels post-administration; B) urine production increase as seen post-administration; C) transient decrease in urine specific gravity following administration. Experiments were performed in five horses and average results are presented. Note: mg/Dl represents mg per deciliter. | *Auswirkungen von Guanabenz auf die Blut- und Urinchemie während des 8-minütigen Zeitraums nach der Injektion von 0,2 mg/kg. A) Blut- und Uringlukosespiegel nach der Verabreichung; B) Anstieg der Urinproduktion nach der Verabreichung; C) vorübergehende Abnahme der spezifischen Uringewichtskraft nach der Verabreichung. Die Experimente wurden an fünf Pferden durchgeführt, und es werden die durchschnittlichen Ergebnisse dargestellt. Anmerkung: mg/Dl steht für mg pro Deziliter.*

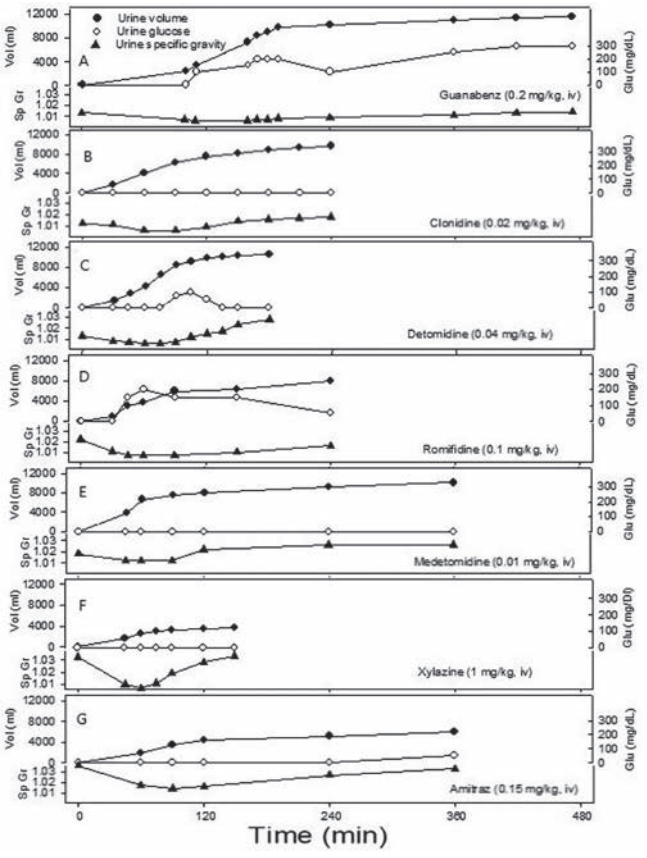

Fig. 7 Cumulative urine volumes, specific gravity, and urine glucose following IV administration of the alpha-2 agonists. Experiments with guanabenz and clonidine were performed with five horses, and average results are shown. Experiments with detomidine, romifidine, medetomidine, xylazine, and amitraz were performed each in a single horse. | *Kumulative Urinmengen, spezifisches Gewicht und Uringlukose nach intravenöser Verabreichung der Alpha-2-Agonisten. Die Versuche mit Guanabenz und Clonidin wurden mit fünf Pferden durchgeführt, und es werden die durchschnittlichen Ergebnisse gezeigt. Versuche mit Detomidin, Romifidin, Medetomidin, Xylazin und Amitraz,wurden jeweils an einem einzigen Pferd durchgeführt.*

pha-2 agonists included in this study for combination with opioids for pain management based on maintaining maximum level of analgesia for longer periods of time.

Analgesia

It is difficult to compare the efficacy of analgesic agents since pain can be superficial, deep, or visceral, and the different types of pain have varying responses to analgesic drugs. For example, xylazine and detomidine are routinely used for the relief of colic (visceral) pain in horses. One study found that romifidine produced limited analgesia for visceral pain in a dose-related manner (*Poulsen* 1988), and another study concluded that the limited analgesic effect was neither dose-related nor consistent (*Voegtli* 1988). One study comparing romifidine and detomidine concluded that the analgesic effects of both agents were similar (*Scrollavezza* et al. 1993); another comparative study concluded that romifidine had no analgesic effect on peripheral pain (*Hamm* et al. 1995). Medetomidine has not been evaluated previously as an analgesic agent in horses, although its analgesic effect has been well demonstrated in other species (*Stenburg* 1989). In a study that used a heat lamp similar to the one described in this paper, amitraz produced analgesia to peripheral pain in a dose-related manner (*Queiroz-Neto* et al. 1995). The lowest administered dose (0.05 mg/kg) produced significant anesthesia 30–45 min post-administration. The highest administered dose (0.15 mg/kg) produced significant analgesia from 5–150 min after administration. In our study, guanabenz produced the longest antinociceptive effect lasting up to 360 min in contrast to effects lasting only 60 min or less for romifidine, xylazine or medetomidine. These four compounds produced similar HWRL effects to > 250 %, as opposed to detomidine and clonidine which produced short duration effects to 60 to 120 min at only 150 % HWRL.

Blood pressure

The effects of guanabenz on blood pressure were not measured here. However, given this compound's prominence as a remedy for hypertension, the following quote is relevant: "In veterinary medical applications, equine racetrack veterinarians have previously used an 8 mg tablet of guanabenz, crushed and dissolved in water, and injected the resultant solution intravenously into horses prior to racing. The rationale behind this administration is that it reduces the blood pressure in the horse's pulmonary circulatory tract and thereby reduces the incidence and/or severity of exercise induced pulmonary hemorrhage (EIPH) in the horse" (*Tobin* 2001). Reduction in heart rate by guanabenz up to about minute 210 (Fig. 5) underscores this hypotensive effect with shorter effects to about 60 min by amitraz, romifidine, clonidine and medetomidine, and very reduced effects if any versus controls for detomidine and xylazine.

Urine chemistry

At ~2.0 hr after guanabenz administration, the transport maximum for glucose was apparently reached in the kidney tubules, and glucose started to appear in the urine (Fig. 7a). A dose-dependent hyperglycemia has been noted following administration of xylazine (*Thurmon* et al. 1982), detomidine (*Gasthuys* et al. 1987), and romifidine (*Gasthuys* et al. 1993, 1996), and elevated levels of urine glucose have been measured in some, but not all, of the studies. The hyperglycemia and the corresponding glucosuria resulted in a significant diuresis, as shown by the cumulative urine volume. The increased urine production has been attributed to an osmotic diuresis from spillover of glucose into the urine for some alpha-2 agonists. However, there is a suggestion that the polyuria is also mediated by an inhibition of arginine vasopressin release (*Kim* et al. 2021), which could explain the increased urine volume following administration of alpha-2 agonists that do not show glucosuria, e.g., medetomidine, clonidine, and xylazine (Fig. 7). *Harada* et al. (1992) noted that the mechanism of the diuretic or anti-diuretic action of alpha-2 adrenergic agents may vary depending on the species. For example, clonidine has been shown to inhibit diuresis by the reduction of anti-diuretic hormone (ADH), to block renal tubular action of ADH, to increase the glomerular filtration rate, or to inhibit renin release.

Limitations of the study

This study has been limited in its applicability by the small number of test subjects, five for guanabenz and clonidine and only one for each of the other compounds. If we apply the Sample Size Calculator available at https://www.calculator.net/sample-size-calculator.html with settings of 95 % confidence, an acceptable margin of error of 25 %, and a population proportion of 10 % of horses requiring treatment, a minimum sample number of 6 is returned, meaning we are one horse short for the guanabenz/clonidine investigation. In practical terms, this may bear mainly on the statistical acceptability of some measured parameters listed in Table 1, such as Head Droop Duration or Sedation as Reduced Locomotor Activity. Note that statistical significance is heavily dependent on the study's sample size; with large sample sizes, even small treatment effects (which may in actuality be clinically inconsequential) can appear statistically significant; therefore, the authors felt that statistical analysis was not relevant in the current study primarily due to sample size ($n = 1$ for most of the alpha-2 agonists), and feel that the figures are sufficient enough to compare especially the analgesic and sedative activities of various alpha-2 agonists in a clinically meaningful matter.

Another limitation is the lack of opportunity for running the data collection in blinded fashion, since the person preparing drugs for administration, making injections and carrying out the measurements was the same person.

Conclusion

Although not compared statistically, these studies strongly suggest that intravenous guanabenz (0.2 mg/kg) induces sedation more rapidly than that produced by other alpha-2 agonists, and that the sedation and analgesic responses were generally more intense and considerably longer-lasting than responses to other members of the alpha-2 agonists group evaluated. These experiments suggest considerable clinical potential for guanabenz as a sedative and as a relatively long-lasting analgesic in equine medicine. Further prospective studies analyzing

the pharmacokinetics and pharmacodynamics of guanabenz in relation to the other alpha-2 agonists are merited.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author's contributions

A. F. Lehner, L. Dirikolu and *T. Tobin* contributed to the analytical development method, validation and sample analysis. *T. Tobin* performed project management and fund raising. *A. F. Lehner*, *L. Dirikolu* and *T. Tobin* performed manuscript editing and *T. Tobin* organized data compilation. All authors contributed to the writing of the manuscript and have read and approved the final manuscript.

Animal ethics statement

The authors confirm that the ethical policies of this journal have been adhered to, and the appropriate ethical review committee approval has been received. Animals used in these experiments were managed in accordance with the rules and regulations of the University of Kentucky Institutional Animal Care Use Committee (IACUC) which also approved the experimental protocol, assigned IACUC number 00137A2000 under the title "Drug Test Development and Validation". The authors confirm that they adhered to the IACUC-approved protocol.

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