In vitro effects of oxytocin, acepromazine, detomidine, xylazine, butorphanol, terbutaline, isoproterenol, and dantrolene on smooth and skeletal muscles of the equine esophagus

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Objective—To characterize the in vitro effects of oxytocin, acepromazine, xylazine, butorphanol, detomidine, dantrolene, isoproterenol, and terbutaline on skeletal and smooth muscle from the equine esophagus.

Animals—14 adult horses without digestive tract disease.

Procedure—Circular and longitudinal strips from the skeletal and smooth muscle of the esophagus were suspended in tissue baths, connected to force-displacement transducers interfaced with a physiograph, and electrical field stimulation was applied. Cumulative concentration-response curves were generated for oxytocin, acepromazine, xylazine, detomidine, butorphanol, isoproterenol, terbutaline, and dantrolene. Mean maximum twitch amplitude for 3 contractions/min was recorded and compared with predrug-vehicle values for the smooth muscle preparations, and the effects were characterized.

Results—No drugs caused a significant change in skeletal muscle response. In smooth muscle, isoproterenol, terbutaline, and oxytocin significantly reduced AUC in a concentration-dependent manner. Maximum reduction in AUC was 69% at 10⁻⁴M for isoproterenol, 63% at 10⁻⁵M for terbutaline, and 64% at 10⁻⁴M for oxytocin.

Conclusions and Clinical Relevance—Isoproterenol, terbutaline, and oxytocin cause relaxation of the smooth muscle portion of the esophagus. The clinical relevance of these effects on the proximal portion of the esophagus reported of drugs such as oxytocin, detomidine, and acepromazine may be the result of centrally mediated mechanisms. (Am J Vet Res 2002;63:1732–1737)

Received Jan 9, 2002. Accepted Jun 4, 2002.

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This report represents a portion of the thesis submitted by the first author to the graduate school, Louisiana State University, in partial fulfillment of the requirements for the MS degree.


The authors thank Catherine Koch and Michael Broussare for technical assistance.

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Esophageal obstruction is the most commonly diagnosed esophageal disorder of horses. Xylazine, detomidine, acepromazine, butorphanol, and oxytocin are used in the treatment of this condition to decrease pain and anxiety, lower the horse’s head, and potentially relax the spasm around the obstruction. The effects of these drugs on equine esophageal muscle have not been critically elucidated in vitro, and there are few evaluations in vivo.

The equine esophagus is composed of skeletal muscle in the proximal two-thirds and smooth muscle in the distal one-third. Most obstructions occur in the skeletal muscle portion at the post-pharyngeal area or at the area of the thoracic inlet. Denac et al. evaluated the effect of various neurotransmitters on smooth muscle preparations of equine esophagus in vitro. Norepinephrine caused concentration-dependent relaxation of longitudinal esophageal smooth muscle from the esophageal body via its agonist effects on β receptors. Acetylcholine and norepinephrine (via α receptors) caused concentration-dependent contraction of the circular smooth muscle of the esophageal body and the lower esophageal sphincter. Electrical field stimulation (EFS) was also applied to the smooth muscle preparations, and the effects were characterized. The skeletal muscle portion of the equine esophagus has not been tested in vitro.

We hypothesized that xylazine, detomidine, oxytocin, butorphanol, acepromazine, and dantrolene would cause relaxation of skeletal esophageal muscle, and isoproterenol and terbutaline would have no effect on skeletal muscle. We also hypothesized that isoproterenol, terbutaline, xylazine, detomidine, acepromazine, and butorphanol would cause relaxation of esophageal smooth muscle, dantrolene would have no effect, and oxytocin would have either no effect or cause contraction. The purpose of the study reported here was to characterize the effects of these drugs on isolated preparations of skeletal and smooth muscle from the equine esophagus.

Materials and Methods

Tissue harvest and preparation—This study was approved by the Louisiana State University Institutional Animal Care and Use Committee. Muscle from the esophageal body was harvested from 14 adult horses that were euthanatized for reasons unrelated to digestive tract disease. Tissue was collected immediately after euthanasia with an overdose of pentobarbital sodium (100 mg/kg, IV). Skeletal esophageal tissue was collected from the cer-
vical region, excluding the upper esophageal sphincter, and smooth muscle was collected from the distal thoracic region, excluding the lower esophageal sphincter. The tissue was immediately placed into warmed, oxygenated Krebs solution and transported to the laboratory. The Krebs solution composition was NaCl (118mM), KCl (4.7mM), NaHCO₃ (64.01 g/L), MgSO₄ (1.2mM), KH₂PO₄ (1.1mM), CaCl₂ × 2 H₂O (2.5mM), and 3.5 g/L of dextrose. The esophagus was opened longitudinally, and the mucosa and submucosa were gently removed using sharp dissec-
tion to expose the muscular layers. The muscle was orient-
ed so that 2 circular and then 2 longitudinal muscle strips, 1.5 × 0.5 cm, were collected from each of the skeletal muscle and smooth muscle portions of the esophagus. The 8 preparations were suspended in individual tissue baths containing 14 mL of Krebs solution, maintained at 37°C, and continuously aerated with 95% O₂ and 5% CO₂. One end of each strip was fixed with silk suture to a force-dis-
placement transducer interfaced with a polygraph, and the other end was fixed to the base of the tissue bath.

Electrical field stimulation—Electrical field stimulation was accomplished by suspending each tissue preparation between 2 platinum wire electrodes mounted on the tissue bath. A square wave electrical impulse was generated by a stimulator and applied to all 8 tissues. Repetitive stimuli were provided at 5 Hz, 0.5 ms for skeletal muscle, and 90 V, 10 Hz, 1 ms for smooth muscle) were applied at a rate of 0.025 trains/s. Pilot studies in our laboratory determined that these parameters resulted in submaximal stimulation for these skeletal and smooth muscle tissues as reported for equine esophageal smooth muscle and rabbit urethral skeletal muscle.

Experimental design—Tissues from 3 horses were evaluated without EFS, and tissues from 11 horses were evalu-
at with EFS. The experiments on unstimulated tissues were performed to evaluate spontaneous activity. Electrical field stimulation was used for more consistent contractions and because a relaxation response could be easily identified and accurately measured.

For evaluation of tissue responses without EFS, smooth and skeletal muscle tissues were allowed to equilibrate in the baths for 1 hour, and then resting tension was set to 1 to 2 g. Tissues were washed with fresh Krebs solution at 15-minute intervals, and the tension was reset to 1 to 2 g at each wash-
ing until the final washing before drug administration. The tissues were first observed for spontaneous activity for 15 minutes. A pilot study was performed on the smooth and skeletal muscle to determine a drug-induction of maximum contraction amplitude. The drugs tested in the pilot study were histamine, neostigmine, norepinephrine, oxytocin, and carbachol. Oxytocin was included with the other drugs because it was expected to cause contraction of smooth muscle, and we wanted to determine its effect on unstimulated esophageal skeletal muscle, given the recent interest in the drug. Cumulative concentrations of histamine (10⁻⁷, 10⁻⁶, 10⁻⁵, 10⁻⁴, 10⁻³ M), KCl (10⁻⁷ M), neostigmine (10⁻⁷, 10⁻⁶, 10⁻⁵, 10⁻⁴, 10⁻³ M), BaCl₂ (10⁻³ M), norepinephrine (10⁻⁷, 10⁻⁶, 10⁻⁵, 10⁻⁴, 10⁻³ M), oxytocin (10⁻⁷, 10⁻⁶, 10⁻⁵, and carbachol (10⁻⁷, 10⁻⁶, 10⁻⁵, 10⁻⁴ M) were individually added to the tissue baths to reach the desired concentration in the bath. Tissues were washed with fresh Krebs solution between drugs. Carbachol was the only drug to have any effect, and it caused a concentration-dependent increase in tension in both smooth and skeletal muscle, with maximum contraction amplitude first occurring at 10⁻⁶ M. After determining a drug-induction of maximum contraction amplitude in the pilot study, an experiment was performed to evaluate a relax-

ant effect of oxytocin after precontraction with carbachol (10⁻⁶ M). After equilibration and establishment of resting baseline of the smooth and skeletal muscle tissues, tension was increased by placing carbachol (10⁻⁶ M) into the bath. When the response plateaued, oxytocin (10⁻⁷, 10⁻⁶, 10⁻⁵, 10⁻⁴ M) or vehicle (water) was added to the tissues to determine a cumulative concentration-response relationship. Parameters measured included baseline, contraction amplitude, and frequency before carbachol administration, after carbachol administration, and for each concentration of oxytocin or vehicle.

Smooth and skeletal muscle tissues from 11 horses were used for EFS. After preparation, the tissues were allowed to equilibrate for 1 hour, and then resting tension was set to 1 to 2 g. Tissues were washed with fresh Krebs solution at 15-minute intervals throughout the experiment. Electrical field stimulation was applied for 15 minutes, and the tissues were washed and rested for another 15 minutes. The drugs tested on the stimulated tissues included oxy-
tocin (10⁻⁷ to 10⁻⁵ M), acromazine (10⁻⁵ to 10⁻⁴ M), isoproterenol (10⁻⁶ to 10⁻⁵ M), terbutaline (10⁻⁵ to 10⁻⁴ M), xylazine (10⁻⁴ to 10⁻³ M), butorphanol (10⁻⁴ to 10⁻³ M), detomi-
dine (10⁻⁴ to 10⁻³ M), dantrolene (10⁻³ to 10⁻² M), and vehicle control (water). For oxytocin, only lower concen-
trations (10⁻⁷ to 10⁻⁶ M) were used for the first 2 horses, and more concentrations (10⁻⁵, 10⁻⁴, and 10⁻³ M) were added for the next 9 horses. This resulted in analysis of more tissues for the lower concentrations of oxytocin in the final analy-

sis. Drugs or vehicle were administered at 1-minute inter-
vals in increasing concentrations; 10 μL of each drug dilu-
tion or 10 μL of vehicle (water) was added to the baths to achieve the final drug concentration in the bath. Electrical field stimulated-contractions were recorded for 1 minute before drug administration. Contractions were measured for 1 minute after each concentration of drug or vehicle was added to the baths. Acromazine was always administered last because of the need for acid-washing the tissue baths after use of this drug. The other drugs or vehicle was administered in random order. Baths were rinsed extensively with Krebs solution 3 times after the highest concentra-
tion of each drug was administered, and the tissues were rested for 15 minutes between drugs. Tetrodotoxin (3 X 10⁻³ M), which blocks sodium channels so that a nerve-stim-
ulated action potential cannot be generated, was adminis-
tered after the final washing to determine that responses to EFS were nerve-mediated.

For skeletal muscle, stimulation caused 3 sets of t−7
chw 0.5 g were

tractions/min. Maximum twitch amplitudes (grams) of the 3 sets were measured with calipers, and the mean was cal-
culated for 1 minute before drug or vehicle administration and for each drug or vehicle concentration. Percentage change from mean maximum twitch amplitude before drug administration was calculated for each drug concentra-
tion or vehicle.

For smooth muscle, stimulation caused 3 sustained con-
tractions (rather than sets of twitches) per minute. Area under the curve (AUC) was the measurement performed on con-
tractions to account for duration as well as maximum contract-
amplitude. This was measured and averaged for 3 con-
tractions for 1 minute before drug or vehicle administration and for each drug concentration or vehicle control. Percentage change from mean AUC before drug or vehicle administration was calculated for each drug concentration or vehicle.

For stimulated smooth and skeletal muscle, tissues with mean maximum contraction or twitch amplitudes before drug or vehicle administration of < 0.5 g were excluded from the experiment because they were considered to be nonviable tissues.

Statistical analyses—The data were found to follow a normal distribution after arcsine transformation by use of
the Shapiro-Wilk statistic, with failure to reject the null hypothesis of normality at $P < 0.05$. The transformed data were analyzed by use of a mixed effect general linear model procedure, PROC MIXED, which accounted for the random variance of horse and repeated measurements on each horse. There was no difference in response of longitudinal versus circular layers, so the data were pooled and reanalyzed with the general linear model procedure. When there were significant effects of drugs at $P < 0.05$, predetermined comparisons from mean maximum twitch amplitude or AUC before drug or vehicle administration and from vehicle controls were made by use of the differences of least squares means. For all comparisons, a value of $P < 0.05$ was considered significant. A significant percentage of change attributable to a drug was considered to occur if there was a significant difference from percentage change before drug or vehicle administration and from the vehicle percentage change occurring at the same time interval as that concentration of drug. Both of these comparisons were made to determine a difference from values before drug or vehicle administration and to control for tissue fatigue over time. All data are reported as mean ± SEM.

Results

**Unstimulated tissues**—Analysis of unstimulated tissues was performed on 4 tissues for oxytocin for smooth muscle and 4 for vehicle control (4 were nonviable), and on 5 tissues for oxytocin for skeletal muscle and 4 for vehicle control (3 were nonviable). The tissues tended to relax after the resting tension was set, and the baseline would return to 0 g of tension. Smooth muscle had spontaneous activity, whereas skeletal muscle did not. Carbachol ($10^{-4}M$) raised the baseline tension, increased frequency of contractions, and increased amplitude of contractions in smooth and skeletal muscle. Baseline tension in skeletal muscle increased from resting baseline of 0.0 g to stimulated baseline of 0.8 ± 0.2 g after administration of carbachol. Carbachol increased the maximum contraction amplitude from 0 to 4.8 ± 2.0 g in skeletal muscle. The frequency of spontaneous contractions in skeletal muscle increased from 0.0 to 4.5 ± 0.4/min after carbachol administration. In smooth muscle, baseline tension increased from 0.25 ± 0.07 g (resting baseline) to 0.95 ± 0.23 g (stimulated baseline) after carbachol administration. Carbachol increased the maximum contraction amplitude from 0.55 ± 0.17 to 4.2 ± 0.78 g in smooth muscle. The frequency of contraction in the smooth muscle increased from 1.75 ± 0.53 to 3 ± 0.35/min after carbachol administration.

There was no difference between tissues precontracted with carbachol and treated with oxytocin versus those treated with a vehicle in either smooth or skeletal muscles.

**Stimulated skeletal muscle strips**—Thirty tissues were analyzed for each drug or vehicle for skeletal muscle (except for the lower concentrations of oxytocin, which used 20 tissues). Fourteen tissues were not included in analysis because they were nonviable. There was no significant response to any of the tested drugs and no evidence of tissue fatigue over time (Fig 1). Tetrodotoxin decreased the response to stimulation, confirming nerve-mediated responses to EFS.

**Stimulated smooth muscle strips**—Twenty-three smooth muscle strips were analyzed for each drug or vehicle for the stimulated muscle experiment (except for the higher concentrations of oxytocin, which used 16). Twenty-one tissues were nonviable. Tetrodotoxin decreased the response to stimulation, confirming a nerve-mediated response to EFS. The AUC for the vehicle-treated tissues at each interval corresponding to increasing concentrations of the drug were all significantly lower than the AUC before vehicle administration, indicating tissue fatigue across time. Isoproterenol, terbutaline, and oxytocin caused a significant decrease in AUC when compared with AUC before drug administration and with the vehicle percentage change corresponding to that concentration of drug, indicating a significant effect of drug. Isoproterenol caused a significant decrease in AUC at concentrations of $10^{-3}M$, $10^{-4}M$, and $10^{-5}M$, and terbutaline caused a decrease in AUC at $10^{-4}M$ (Fig 1 and 2). The maximum decrease in AUC for isoproterenol was 69% at $10^{-3}M$, and for terbutaline was 63% at $10^{-3}M$. Oxytocin caused a decrease in AUC at $10^{-3}$, $10^{-4}$, and $10^{-5}M$, and the maximum decrease was 64% at $10^{-4}M$ (Fig 3). Xylazine, acepromazine, butorphanol, detomidine, and dantrolene did not cause a significant decrease in AUC at any drug concentration.

![Figure 1](https://example.com/fig1.png)

**Figure 1**—A—Response (maximum twitch amplitude [g]) of electrical field stimulated (EFS) skeletal esophageal muscle to increasing concentrations of oxytocin (Oxy). Notice a decrease in AUC in response to oxytocin (Oxy) and no tissue fatigue over time. B—Response (area under the curve [AUC]) of EFS esophageal smooth muscle to increasing concentrations of isoproterenol (Iso). Notice no change in amplitude in response to oxytocin (Oxy) and no tissue fatigue over time.
We hypothesized that oxytocin, detomidine, xylazine, butorphanol, acepromazine, and dantrolene would cause a decrease in maximum twitch amplitude in skeletal esophageal muscle. We expected oxytocin, a peptide hypothalamic hormone, to cause a decrease in maximum twitch amplitude in the skeletal muscle, because it was reported in 2 in vivo studies that oxytocin caused a pressure decrease and clinical resolution of luminal obstructions in the proximal esophagus.19 Because oxytocin did not cause a decrease in maximum amplitude in our model in vitro, the effect observed in vivo could be due to a centrally mediated mechanism rather than a local mechanism. The smooth muscle relaxation caused by oxytocin could potentially cause the skeletal muscle to relieve pressure as well as to open the passageway for food boluses to pass. Oxytocin has been implicated as a neurotransmitter or neuromodulating agent and affects nociception in the spinal cord.10 Esophageal skeletal muscle activity is controlled by nerve-mediated stimulation from the brain, causing stimulation of nicotinic receptors via the vagus nerve.11 Oxytocin could be acting as a neuromodulator of the central nerve-stimulated activity of the proximal portion of the esophagus in vivo.

Dantrolene was expected to cause a decrease in maximum twitch amplitude in the skeletal muscle of the esophagus in vitro, because it is a direct-acting skeletal muscle relaxant that interferes with excitation-contraction coupling at the sarcoplasmic reticulum and at the T-tubule system by changing calcium release and reuptake.12 The effects of dantrolene on the equine esophagus have not been studied, but have been studied in vitro on sections of skeletal muscle from the external urethral sphincter in rats and humans.7 Dantrolene did cause a decrease in nerve-mediated (tetrodotoxin sensitive) twitch amplitude in vitro in the external urethral sphincter and had no effect on the smooth muscle of the bladder in rats.7 Dantrolene had no effect on the skeletal muscle in our study, possibly because the effect on skeletal esophageal muscle is different from that on the external urethral sphincter, or because conditions (eg, pH, calcium concentration) in the bath may not have been optimal for an effect. Because skeletal muscle in the external urethral sphincter is interlaced with smooth muscle and the innervation to that area is complex, responses to drugs could be quite different from responses in esophageal skeletal muscle.7

Detomidine and xylazine, α-2 receptor agonists, had no effect on skeletal muscle in vitro. Detomidine has profound inhibitory effects on motility of the proximal portion of the esophagus in horses when studied with contrast radiography in vivo.3,11 Neither of these drugs has been evaluated on skeletal muscle of the esophagus in vitro, but a nonspecific α-receptor blocking agent, phentolamine, has been evaluated on the external urethral sphincter in vitro in rats and had no effect, suggesting that local α-receptors are not important in the stimulation or relaxation of that skeletal muscle in vitro.7 The effects of detomidine observed with contrast radiography in vivo may be through a central, rather than a locally, mediated mechanism, which explains the lack of response in the skeletal muscle seen in vitro. Neither butorphanol, an opiate agonist-antagonist, nor acepromazine, a phenothiazine tranquilizer, has been reported to cause much effect on...
esophageal motility in vivo, but are reported to be clinically useful.\textsuperscript{11,13} Neither drug caused any changes in the skeletal muscle in vitro, so the effects observed clinically may be from pain and anxiety relief rather than from direct relaxation of the esophageal muscle.

The smooth muscle of the equine esophagus is arranged in circular and longitudinal layers that contain an intrinsic nerve plexus, which plays a role in regulation of motility.\textsuperscript{2,6} Smooth muscle contraction activity is controlled by nerve-mediated stimulation from the intrinsic nerve plexus of nerves in the esophageal wall.\textsuperscript{2,6} Our in vitro study concentrated on drugs that affect the muscular contraction of the aorta, basilar arteries, and cremaster striated components of the urinary bladder outlet.\textsuperscript{2,24} This vasodilatory response is probably associated with nitric oxide release or V1-vasopressinergic receptor stimulation.\textsuperscript{2,24} Oxytocin caused a decrease in tension in the smooth muscle in our study, which could have contributed to the beneficial effects of oxytocin in the treatment of proximal obstructions observed in vivo, potentially by opening the distal passageway for the obstruction to pass, which lessens the workload of the proximal portion of the esophagus.\textsuperscript{12,13} A mechanism of action was not elucidated, but may be mediated by nitric oxide release or the V1-vasopressinergic receptor stimulation, as in some vessels.

No drugs tested in vitro had a significant effect on the proximal portion of the esophagus. The clinical effects of drugs such as oxytocin, detomidine, xylazine, acepromazine, and butorphanol may be attributable to centrally mediated relaxation of the esophagus. The sedatives and analgesics may also be causing relief of pain and anxiety rather than indirectly relaxing esophageal spasm.

The relaxant effect of isoproterenol, terbutaline, and oxytocin on smooth muscle could be clinically useful with distal obstructions, although most obstructions occur in the proximal portion of the equine esophagus.

\begin{itemize}
\item Beuthanasia-D Special, Schering-Plough Animal Health Corp, Kenilworth, NJ.
\item Grass F-D transducer, Grass Instruments Co, Quincy, Mass.
\item Grass model 79 physiograph, Grass Instruments Co, Quincy, Mass.
\item Grass S+8 stimulator, Astro-Med Inc, Grass Instruments Division, West Warwick, RI.
\item Histamine, Sigma-Aldrich Inc, St Louis, Mo.
\item KCL, Sigma-Aldrich Inc, St Louis, Mo.
\item Neostigmine, Sigma-Aldrich Inc, St Louis, Mo.
\item BaCl\(_2\), Sigma-Aldrich Inc, St Louis, Mo.
\item Norepinephrine, Sigma-Aldrich Inc, St Louis, Mo.
\item Oxytocin, Sigma-Aldrich Inc, St Louis, Mo.
\item Carbachol, Sigma-Aldrich Inc, St Louis, Mo.
\item Dantrolene sodium, Procter and Gamble Pharmaceuticals, Cincinnati, Ohio.
\item Tetrodotoxin, Sigma-Aldrich Inc, St Louis, Mo.
\item Sigma Scan Pro 4.0, Jandel Scientific Software, San Rafael, Calif.
\item \`{S}AS V.8.0, \`{S}AS Institute, Cary, NC.
\end{itemize}

References


