97-14-137
#231

Characterization of the Antinociceptive and Sedative Effect of Amitraz in Horses

A. Queiroz-Neto[1†], G. Zamur[1], S.C. Gonçalves[1], A. B. Carregaro[1], M.I. Mataqueiro[1], J.D. Harkins[2] and T. Tobin[2]

[1] Faculdade de Ciências Agrárias e Veterinárias, Câmpus de Jaboticabal, UNESP. 14870-000, Jaboticabal, SP, Brazil. Fax: 016 322 4275

[2] Gluck Equine Research Center, University of Kentucky, Lexington, KY, EUA

[†] To whom all correspondence should be sent

Abstract

Amitraz, an acaricide used to control ectoparasites in animals has a complex pharmacological activity, including α_2-adrenergic agonist action. The purpose of this research was to investigate the possible antinociceptive and/or sedative effect of amitraz in horses. The sedative effect of dimethylformamide (DMF, 5 mL, control) or amitraz (0.05, 0.10, 0.15 mg/kg) was investigated on the head ptosis test. To measure the antinociception, xylazine hydrochloride (1 mg/kg, positive control) and the same doses of amitraz and DMF were used. A focused radiant light/heat directed onto the fetlock and withers of a horse were used as a noxious stimulus to measure the hoof withdrawal reflex latency (HWRL) and the skin twitch reflex latency (STRL). The three doses of amitraz used (0.05, 0.10,and 0.15 mg/kg) provoked a dose-dependent relaxation of the cervical muscles. The experiments with amitraz and xylazine on the HWRL showed that after iv administration of all doses of amitraz there was a significant increase of HWRL up to 150 min after the injections. Additionally, there was a significant difference between control (DMF) and positive control (xylazine) values up to 30 min after drug injection. On the other hand, the experiments on the STRL show that after administration of amitraz at the dose of 0.15 mg/kg, a significant increase in STRL was observed when compared with the control group. This effect lasted up to 120 min after injection. However, no significant antinociceptive effect was observed with the 0.05 and 0.10 mg/kg doses of amitraz or at the 1.0 mg/kg dose of xylazine.

1. Introduction

Amitraz [N-methyl-N'-2,4-xilyl-N-(N-2,4-xilylformidyl) formamide] (AMZ) is an acaricide formamide widely used in the control of ectoparasites in veterinary medicine.

One of the first reports of the use of AMZ in domestic animals was the paper by HARRISON et al. (1973) which showed that this drug is a good alternative for the control of ectoparasites in cattle, especially for the treatment of tick strains resistant to organochlorine and organophosphorus pesticides.

The use of AMZ in horses is contraindicated (SMITH, 1994) because of the common occurrence of severe colic when horses are sprayed with this acaricide. However, in some tropical countries such as Brazil, AMZ continues to be widely used in horses because it is a highly effective and economically interesting tick killer.

In addition to having an excellent acaricidal and insecticidal activity, AMZ has a highly complex pharmacological activity in mammals. In several animal species the most common signs and symptoms of acute intoxication with this pesticide are depression of the central nervous system (CNS) with episodes of hyperexcitability, hypotension, bradycardia, hypothermia, hyperglycemia and, in some species, alterations in water balance demonstrated by blood concentration (BONSALL & TURNBULL, 1983). Furthermore, reduced smooth muscle activity, especially in the digestive tract has been reported, as demonstrated by HSU & McNEEL (1985) in an experiment in which they evaluated intestinal transit by the progression of barium sulfate through the stomach and duodenum of dogs treated with AMZ.

rational use of this substance so as to minimize or even prevent the occurrence of undesirable symptoms in domestic animals, especially horses.

Another point to be considered is the potential therapeutic use of this drug. Like xylazine, detomidine and romidifine, drugs widely used as tranquilizers and/or analgesics for horses, whose effects are mediated by α_2 adrenergic receptors, AMZ is also an α_2 adrenergic agonist but has never been tested in horses in terms of its possible analgesic and/or sedative effect, although several investigators have reported signs of CNS depression after the use of the drug in several animal species.

The objective of the present study was to investigate the sedative and antinociceptive effects of iv administration of AMZ on thoroughbred (TB) horses and to establish a possible correlation of these effects with stimulation of α_2 adrenergic receptors.

2. Material and Methods

The study was conducted on 8 TB mares from the herd of the Faculdade de Ciências Agrarias e Veterinarias of Jaboticabal, UNESP, and on 11 TB mares belonging to the experimental herd of the Department of Veterinary Sciences, University of Kentucky, Lexington, KY, USA.

Sedation was evaluated by the method of head ptosis in 6 animals. After intravenous administration of 5 mL dimethylformamide (DMF, control) or 0.05, 0.1 and 0.15 mg/kg AMZ, the distance from the ground to the lower lip of the animals was measured with the aid of a ruler fixed to the wall at preestablished intervals. These

measurements were repeated up to 360 minutes or until the time when the horse showed normal behavior, demonstrating the end of the effect of the drug.

Nociception and the time and dose-response relationships for AMZ and xylazine were evaluated in the animals with the aid of a heat projection lamp adapted according to the description of KAMERLING et al. (1985a,b) and built by the Electrical Engineering Department of the University of Kentucky, USA.

Rapid exposure to the heat lamp was used as a painful stimulus applied to the following sites in independent experiments:

- region of the lateral surface of the proximal phalanx of the thoracic limb of the horse in order to trigger the classical hoof withdrawal reflex, when we measured the latency to the hoof withdrawal reflex (HWRL), defined as the time between focusing of the light beam and limb withdrawal.

- Region of the withers of the horse in order to measure the latency to the skin twitch reflex (STRL) which represent the time from the beginning of the painful stimulus to the occurrence of skin twitch.

For the above experiments, before applying the focused light, we shaved the region and painted the skin with black water-based ink in order to obtain uniform light reflection and consequently uniform heat absorption.

In both types of experiment, the cut-off time of exposure to the painful stimulus was 10 seconds in order to prevent tissue injury.

A secondary, non-focused lamp was frequently used to confound the animal and to reduce the possible occurrence of the reflex as a conditioned response to light perception rather than to the heat perception caused by the focused light beam.

Both HWRL and STRL were measured at the moments -20, -10 minutes and immediately before iv injection of DMF (5.0 mL, control), xylazine (1 mg/kg, positive control) and AMZ (0.05, 0.10 and 0.15 mg/kg). These three latency measurements obtained before administration of the drugs were used to establish control values for each horse. The latencies were also measured at 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 minutes after drug administration.

The areas under the curve presented in the figures where we demonstrate the dose-response curves for the effects of AMZ in the HWRL, STRL and head ptosis tests were inferred by weighing these areas printed on good quality paper.

Data were analyzed statistically by analysis of variance followed by the Tukey test, with the level of significance set at 5%.

3. Results

Effect of AMZ on head ptosis in horses

The results obtained in this experiment demonstrated that AMZ produced consistent effects in this experimental model of sedation.

Analysis of Figure 1 and table 1 shows that the three doses of AMZ used provoked relaxation of the cervical muscles, which are responsible for maintaining the animal's head in the supine position, causing a marked head ptosis. Figure 2 shows that the effect of AMZ was dose dependent.

Antinociceptive effect of AMZ

The antinociceptive effect of AMZ and xylazine, evaluated by the increase in HWRL, is illustrated in Figure 3 and Table 2.

After iv administration of AMZ at the doses of 0.05, 0.10 and 0.15 mg/kg or of xylazine at the dose of 1 mg/kg (positive control), a significant increase in HWRL was observed compared to the control group injected with DMF. In the group that received the highest dose of AMZ (0.15 mg/kg), the effect was observed until 150 minutes after injection. Figure 4 shows the dose-response curve for the antinociceptive effect of AMZ in the HWRL test.

The antinociceptive effect of AMZ and xylazine, evaluated by the increased STRL, can be seen in Figure 5 and Table 3.

After iv administration of AMZ at the dose of 0.15 mg/kg, a significant increase in STRL was observed compared to the control group injected with DMF and the effect lasted up to 120 minutes after injection. However, no significant antinociceptive effect was observed with the 0.05 and 0.1 mg/kg doses of AMZ or at the 1.0 mg/kg dose of xylazine.

Figure 6 shows the dose-response curve for the antinociceptive effect of AMZ in the STRL test.

4. Discussion

The sedative effect of AMZ in horses was evaluated by determining head ptosis. Relaxation of muscle tonus in the neck is normally observed during sleep. Sedation has been quantitated by the measurement of the degree of head ptosis after administration of tranquilizing/sedative drugs (KAMERLING et al., 1988). Figure 1 and Table 1 clarifie that this method proved to be adequate to measure the sedation provoked by the three AMZ doses used. However, it can be seen that the maximum responses induced by the different doses showed relatively little variation. For the lowest dose, the maximum head ptosis occurred 15 minutes after injection, reaching 69 cm on average, whereas for the intermediate and the highest doses, the maximum effects occurred 30 minutes after administration, reaching 81 and 86 cm, respectively. It can be seen that these mean values were not significantly different from one another but differed significantly from the mean value for the control (Tukey test, $P<0.05$). Also, as observed in the tests for the evaluation of locomotor activity (HARKINS et al, 1997), the duration of the effect was greater with increasing doses, with a good dose-effect relationship (Figure 2).

Based on the pioneering work of HARDY et al. (1940), who developed a method to measure the pain threshold using the heat of a concentrated light beam as the painful stimulus, several investigators started to use the latency to leg withdrawal for the study of analgesics in several animal species. In horses, the perception of pain, as well as the effect of drugs on it, are difficult to measure by this method because these parameters may be affected by the increase or decrease in locomotor activity that

occurs, for example, after the administration of medications such as narcotic analgesics and phenothiazine tranquilizers, respectively. On this basis, KAMERLING et al. (1985a) developed a method of pain perception that is not influenced by the locomotor activity of the animal. With the aid of a support, the heat-producing device is fixed on the horse so that the light beam points at the withers region. In this experiment, the authors demonstrated the adequacy of the method for the measurement of the analgesic effect of fentanyl, a drug known to have a potent analgesic effect and also to increase the locomotor activity of these animals.

In the present experiments, two methods were used to investigate the antinociceptive effect of AMZ, i.e., the hoof withdrawal test and the skin twich test, the latter an adaptation of the method described by KAMERLING et al. (1985b).

In the hoof withdrawal test, the HWRL was measured in animals injected with DMF (vehicle, control) and in animals injected iv with AMZ (0.5, 0.10 and 0.15 mg/kg) or xylazine (Rompum®, 1 mg/kg). Analysis of Figure 3 reveals the intense and short-lasting effect of xylazine, which was maximal at about 10 minutes after administration and did not last more than 30 minutes, as can be seen in more detail in Table 2. In contrast, the effect of AMZ was less intense at the doses used, but longer lasting. Analysis of Table 2 shows that, at the dose of 0.05 mg/kg, AMZ had an inconsistent effect although statistical analysis showed a significant increase in HWRL at 30 and 40 minutes after injection compared to control. On the other hand, comparison of the effects obtained with the administration of the two AMZ doses showed that the 0.15 mg/kg dose had an apparently more intense effect during the first 30 minutes after injection and lasted a little longer when compared to the dose of 0.10 mg/kg. However,

Figure 4 suggests that a linear dose-response relationship existed for the AMZ doses administered.

Evaluation of antinociception in horses using the STRL method yielded different results in terms of the effect of xylazine. Figure 5 shows that, contrary to the results obtained with the limb withdrawal test, the effect of xylazine was of low intensity and short lasted, with no significant variation in STRL after administration of the drug (Table 3). Similarly, Figure 5 and Table 3 suggest that, at the doses of 0.05 and 0.10 mg/kg, AMZ did not present a good antinociceptive effect in terms of an increase in STRL. At the dose of 0.15 mg/kg, however, it was possible to observe a consistent and prolonged antinociceptive effect that was statistically significant from 5 to 150 minutes after administration of the drug. Figure 6 illustrates the inconsistency of the dose-response relationship observed for AMZ in this experimental model.

The results obtained with xylazine suggest the presence of an interaction between the analgesic and sedative effect of drugs. In the experiment of evaluation of antinociception by the HWRL test, in which the locomotor apparatus was of greater importance, the effect of xylazine could be observed. In contrast, in the experiments in which the method for the detection of antinociceptive activity was not related to locomotor activity (STRL), no antinociceptive effect was observed for this drug.

These results agree with those reported by HAMM et al. (1995) who, in a study with detomidine and romifidine administered to horses, surprisingly observed a complete lack of analgesic effect after the administration of romifidine.

Clinicians, and often anesthesiologists, believe that deep sedation may involve a certain degree of analgesia, but the results obtained here with xylazine and those

obtained by HAMM et al. (1995) with romifidine demonstrate that, depending on the drug, this is not always the case. This is an important observation that should serve as an alert to researchers, clinicians, surgeons, anesthesiologists or even other professionals who work with pain, since the fact than an animal does not react to pain does not always mean that it is not feeling anything. What may happen is that the method used to assess pain may not be the most adequate one.

In view of the results obtained and of the considerations about the sedative and antinociceptive effects of α_2 adrenergic agonists in general and of AMZ in particular, we may conclude that this substance presented a marked, long-lasting and powerful sedative effect in horses compared to xylazine. On the other hand, the antinociceptive effects determined by increased STRL was only observed at the highest AMZ dose used, indicating that the major effect of AMZ is sedative and not antinociceptive.

Over the last few years, the interest in the analgesic effects of α_2 adrenergic agonists has greatly increased since they do not cause the side effects of opioid compounds which, according to MAZE and TRANQUILLI (1991), are mainly represented by respiratory depression and potential abuse. Unfortunately, the antinociceptive effect of α_2 adrenergic agonists occurs at doses that also lead to sedation. In this respect, the study by PERTOVAARA et al. (1994), who located the origin of the two effects at different sites in the nervous system, represents a significant contribution. Furthermore, the evaluation of new α_2 adrenergic agonists such as AMZ may lead to the discovery of specific agonists for the different subtypes of α_2 adrenergic receptors, which would represent an important step in the development of more potent drugs with milder side effects.

5. References

AUER, D.E.; SEAWRIGHT, A. A. ; POLLITT, C.C. ; WILLIAMS, G. Illness in horses following spraying with amitraz. **Aust. Vet. J.** 61 (8): 257-9, 1984.

AZIZ, S.A.; KNOWLWS, C.O. Inhibition of monoamine oxidase by the pesticide chlordimeform and related compounds. **Nature** 242: 417-8, 1973.

BENEZET, H.J.; KNOWLES, C.O. Inhibition of rat brain monoamine oxidase by formamidines and related compounds. **Int. J. Neuropharmacol.** 15: 369-73, 1976.

BONSALL. S.L.; TURNBULL, G. J. Extrapolation from safety data to management of poisoning with reference to amitraz (a formamidine pesticide) and xylene. **Hum. Toxicol.** 2 (4): 587, 1983.

CHINN, C.; LUND, A.E.; YIM, G.K.W. Central action of lidocaine and the pesticide chlordimeform. **Neuropharmacol.** 16: 867-71, 1977.

COSTA, L.G.; MURPHY, S.D. Interaction of the pesticide chlordimeform with adrenergic receptors in mouse brain: na *in vitro* study. **Arch. Toxicol.** 59: 323-7, 1987.

GILBERT, M.E. Formamidine pesticides enhance susceptibility to kindled seizures in amygdala and hippocampus of the rat. **Neurotoxicol. Teratol.** 10: 221-7, 1988.

GILBERT, M.E.; MACK, C.M. Enhanced susceptibility to kindling by chlordimeform may be mediated by a local anesthetic action. **Psychopharmacol.** 99: 163-7, 1989.

HAMM, D.; TURCHI, P.; JÖCHLE, W. Sedative and analgesic effects of detomidine and romifidine in horses. **Vet. Rec.** 136: 324-7, 1995.

HARDY, J.D.; WOLFF, H.G.; GOODELL, H. Studies on pain. A new method for measuring pain threshold. Observations on spatial summations of pain. **J. Clin. Invest.** 19: 640-57, 1940.

HARKINS, J.D.; QUEIROZ-NETO, A.; CHEUNG, T.; MUNDY, G.D.; WEST, D.; TOBIN, T. Quantitation of the locomotor responses to therapeutic agents in a behavior chamber and their relationship to other behavioral effects. **J. vet. Pharmacol. Therap.** (accepted for publication on 1997). ??????

HARRISON, I.R.; PALMER, B.H. ; WILMSHURST, E.C. Chemical control of cattle ticks - Resistance problems. **Pest. Sci.** 4: 531-42, 1973.

HSU, W.H. ; McNEEL, S.V. Amitraz-induced prolongation of gastrointestinal transit and bradycardia in dogs and their antagonism by yohimbine: preliminary study. **Drug Chem. Toxicol.** 8 (4): 239-53, 1985.

KAMERLING, S.G.; WECKMAN, T.J.; DEQUICK, D.J. ; TOBIN, T. A method for studying cutaneous pain perception and analgesia in horses. **J. Pharmacol. Meth.** 13: 267, 1985a.

KAMERLING, S.G.; DEQUICK, D.J.; WECKMAN, T.J.; TOBIN, T. Dose-related effects of fentanyl on autonomic and behavioral responses in performance horse. **Gen. Pharmacol.** 16: 253-8, 1985b

KAMERLING, S.G.; CRAVENS, W.M.T. ; BAGWELL, C.A. Objective assessment of detomidine-induced analgesia and sedation in the horse. **Eur. J. Pharmacol.** 151: 1, 1988.

MAZE, M.; TRANQUILLI, W. Alpha-2 adrenoceptor agonists: Defining the role in clinical anesthesia. **Anesthesiology** 74: 581-605, 1991.

PERTOVAARA, A. ; HÄMÄLÄINEN, M.N.; KAUPPILA, T.; MECKE, E.; CARLSON, S. Dissociation of the α_2-adrenergic antinociception from sedation following microinjection of medetomidine into the locus coeruleus in rats. **Pain** 57: 207-15, 1994.

SMITH, S.E. Treatment of mange in horses. **Vet. Rec.** 134 (19): 508, 1994.

YIM, G.K.W.; HOLSAPPLE, M.P.; PFISTER, W.R.; HOLLINGWORTH, R.M. Prostaglandin synthesis inhibited by formamidine pesticides. **Life Sci.** 23: 2509-16, 1978.

Equine Head Ptosis

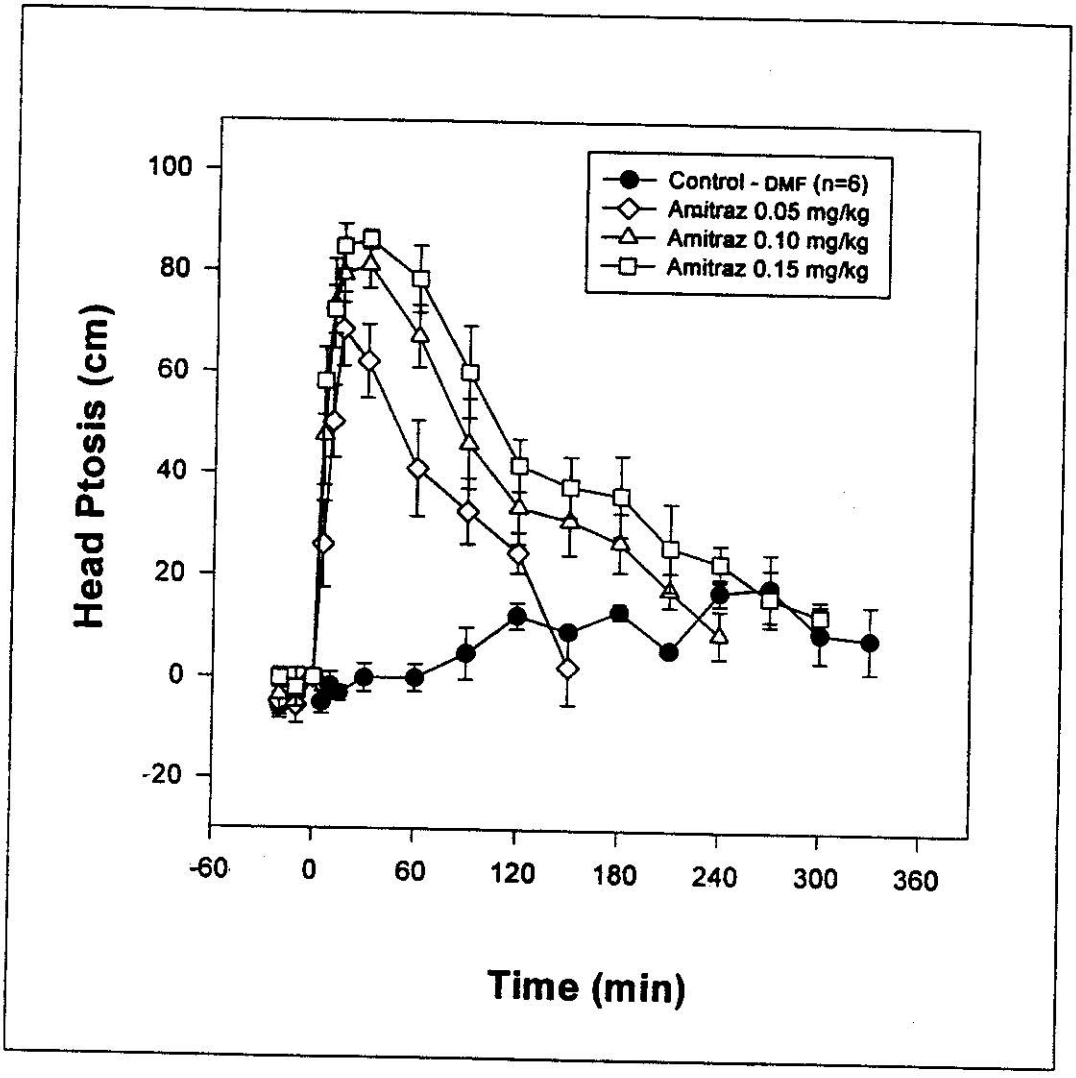

Figure1. Efffect of intravenous administration of dimethylformamide (control) and amitraz (0.05, 0.10 and 0.15 mg/kg) on equine head ptosis. The vertical bars indicate SEM.

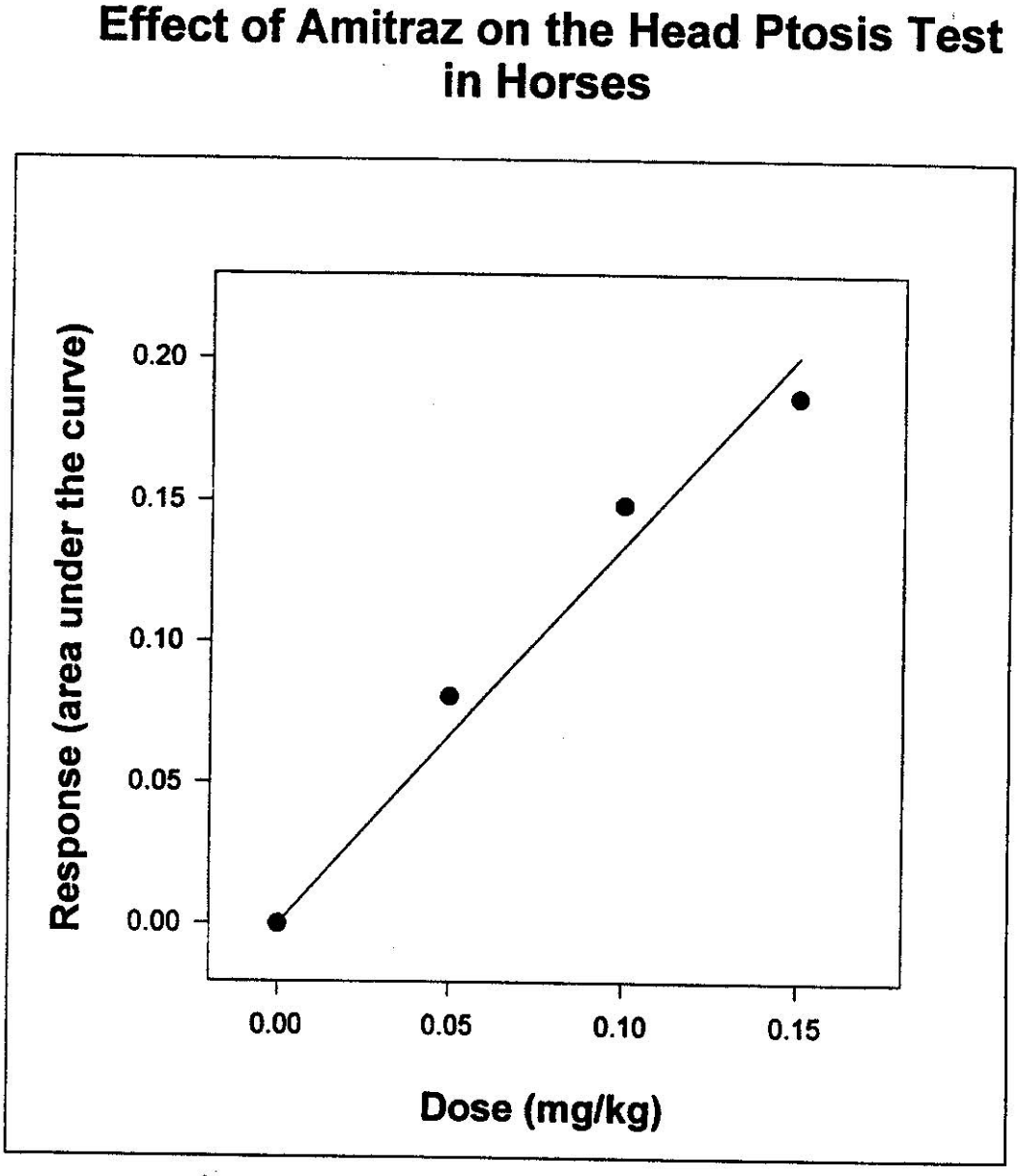

Figure 2. Dose-Response Curve for the sedative effect of amitraz observed by the head ptosis test in horses.

Nociceptive Threshold

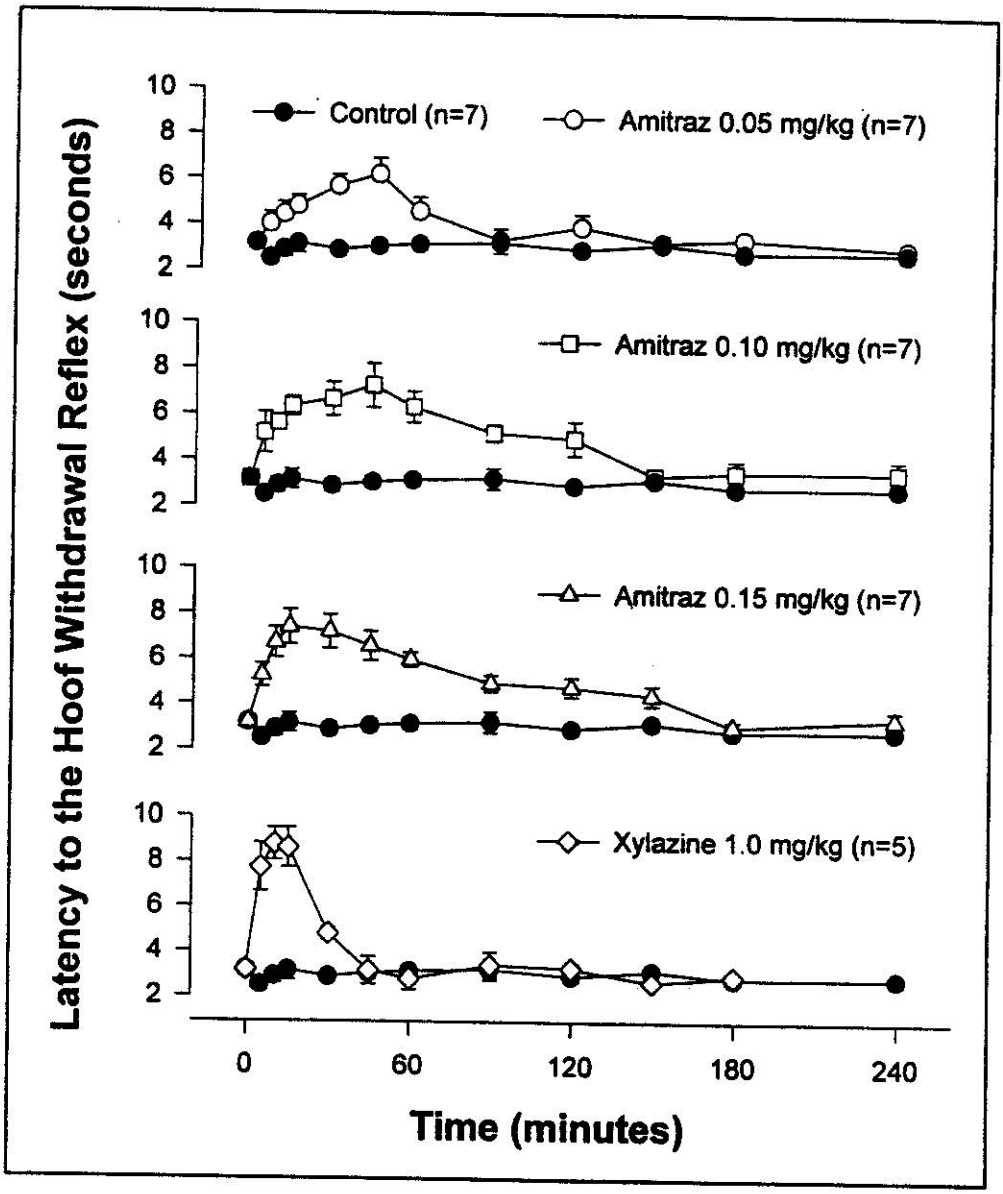

Figure 3. Hoof withdrawal reflex latency (HWRL) in hose after administration of amitraz at the doses of 0.05 ; 0.10 and 0.15 mg/kg, or xylazine at the dose of 1.0 mg/kg. The vertical bars indicate SEM.

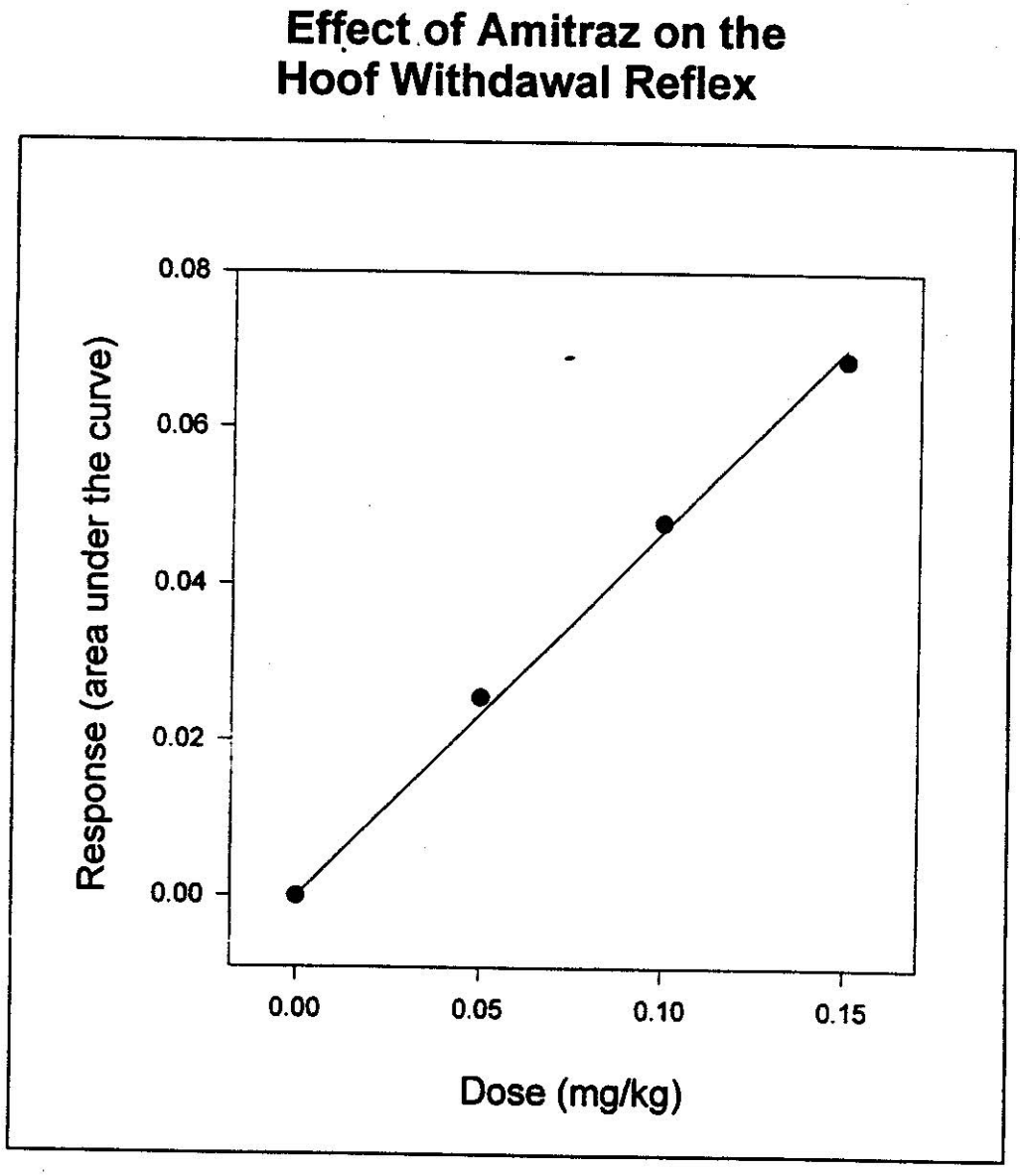

Figure 4. Dose-Response Curve for the antinociceptive effect of amitraz observed as the increase in the hoof withdrawal reflex latency in horses.

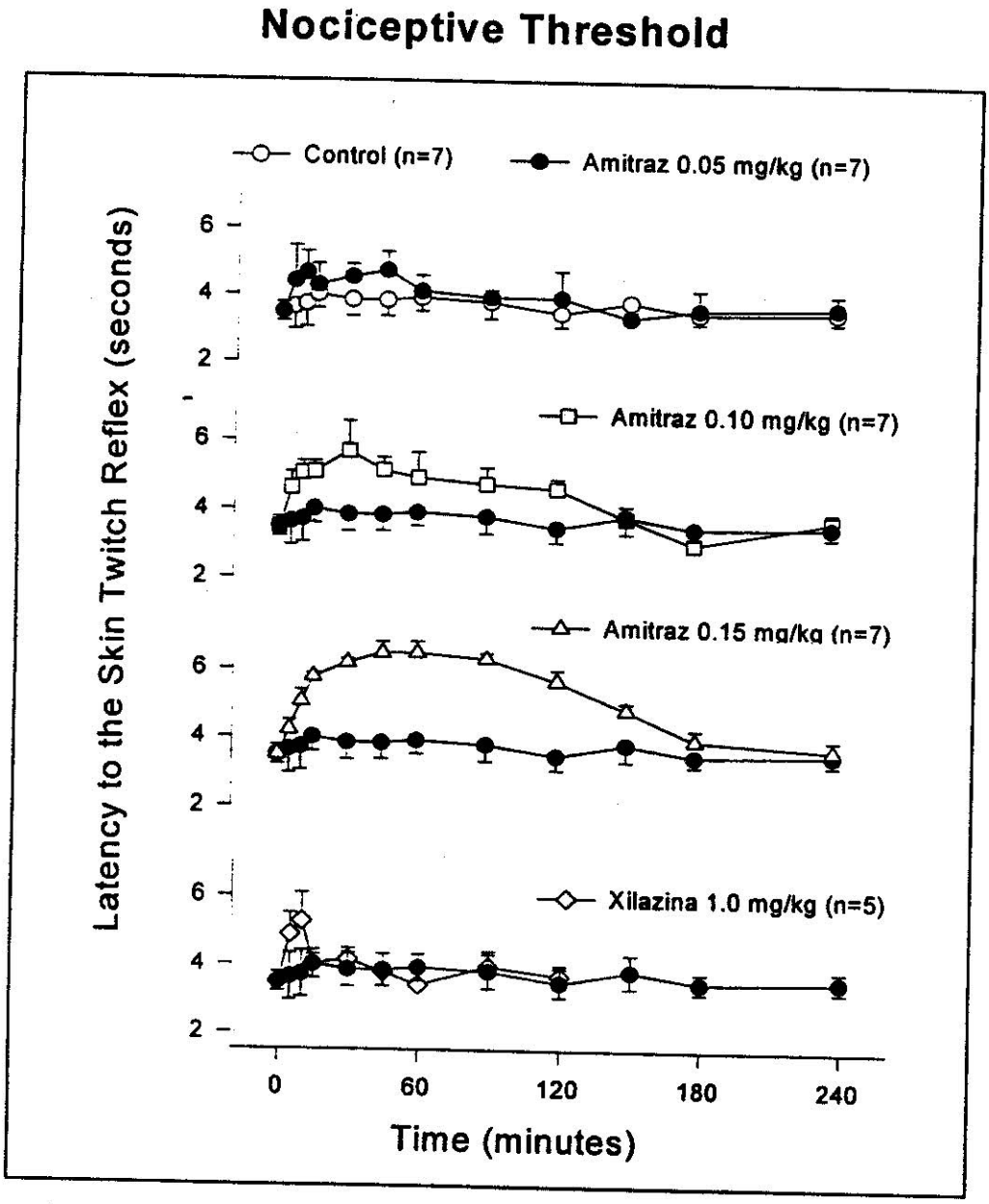

Figure 5. Skin twitch reflex latency (STRL) in horses after intravenous administration of amitraz at the doses of 0.04; 0.10 and 0.15 mg/kg, or of xylazine at the dose of 1.0mg/kg. The vertical bars indicate SEM.

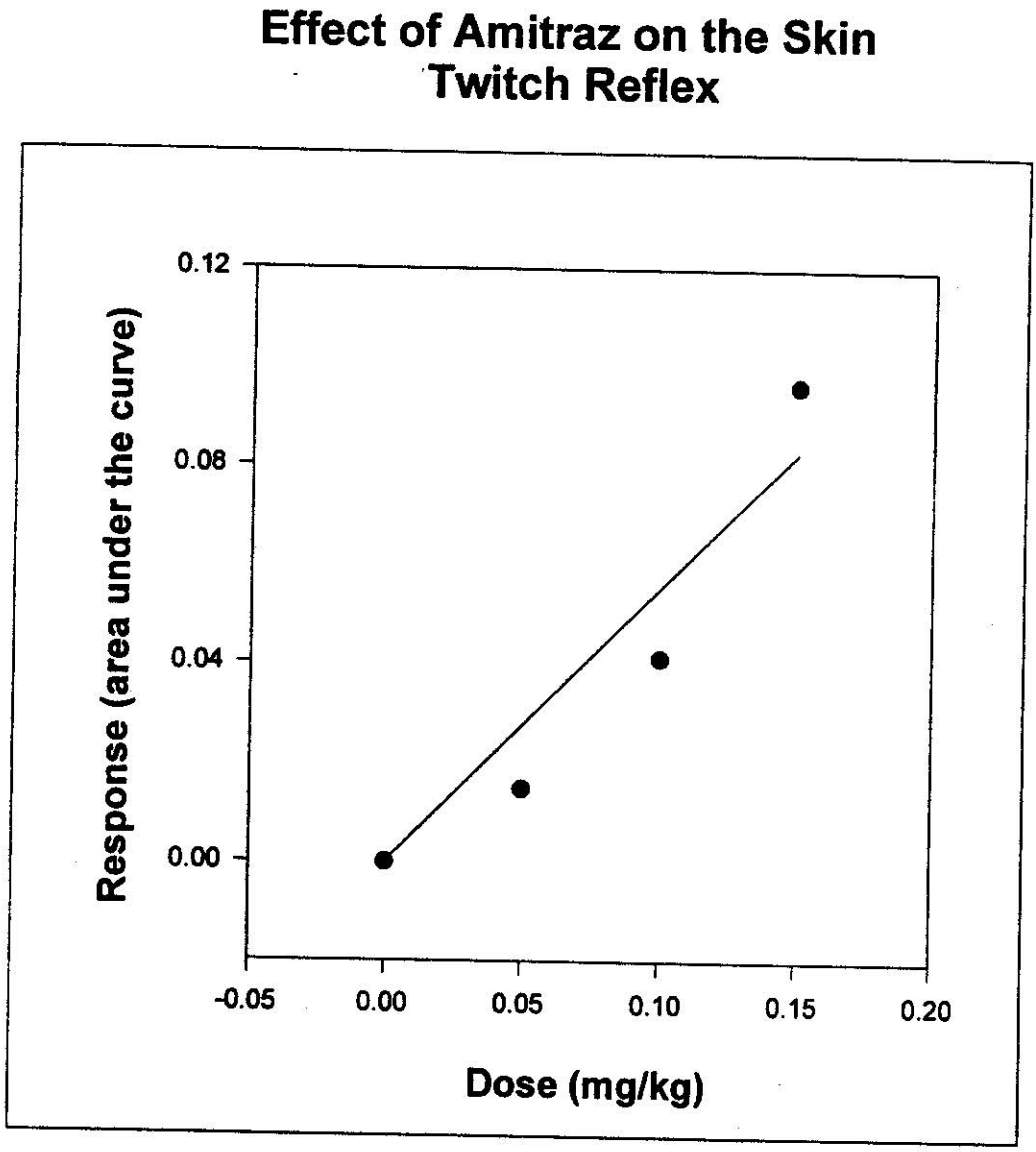

Figura 6. Dose-Response Curve for the antinociceptive effect of Amitraz, observed by the increase in the skin twitch reflex latency (STRL) in horses.

Table 1.

HEAD PTOSIS

DRUG INJECTION → TIME AFTER DRUG ADMINISTRATION (MINUTES)

group	n	-20	-10	5	10	15	30	60	90	120	150	180	210	240	300
Control DMF 5ml	8	-6.62a ± 2.66	-4.99a ± 2.14	-4.74a ± 2.34	-1.87a ± 2.67	-3.24a ± 1.61	1.26a ± 2.67	-0.37a ± 2.78	6.44a ± 1.87	13.69a ± 0.99	9.69ab ± 0.23	12.3ab ± 1.19	5.72a ± 0.34	17.92a ± 0.97	10.52a ± 2.10
AMZ 0.05mg/kg	8	-5.62a ± 2.57	-7.49a ± 2.91	19.51a ± 7.21	48.13b ± 5.86	66.26b ± 6.17	49.63b ± 7.30	36.26b ± 7.91	30.0ab ± 5.52	24.0ab ± 2.96	0.13a ± 6.77	3.31a ± 1.18			
AMZ 0.10mg/kg	8	-3.37a ± 2.95	-3.12a ± 2.49	54.47b ± 8.38	76.88c ± 6.85	81.26b ± 4.28	86.26c ± 4.46	70.88c ± 5.28	48.7bc ± 6.92	36.88b ± 6.13	31.8bc ± 5.05	27.51b ± 5.79	18.7a ± 3.00	11.01a ± 4.42	
AMZ 0.15mg/kg	8	-0.75a ± 2.29	-3.35a ± 4.44	59.25b ± 7.94	73.65c ± 5.41	83.65b ± 5.30	79.65c ± 5.09	80.67c ± 7.42	63.65c ± 10.14	41.65b ± 6.30	38.65c ± 7.03	36.65b ± 9.59	27.65 ± 10.13	23.05a ± 4.23	12.65a ± 2.97

Table 2.

LATENCY TO THE HOOF WITHDRAWAL REFLEX (SECONDS)

DRUG INJECTION ↓		TIME AFTER DRUG ADMINISTRATION (MINUTES)											
group	**n**	**0**	**5**	**10**	**15**	**30**	**45**	**60**	**90**	**120**	**150**	**180**	**240**	**300**
Control DMF 5ml	7	3,18 ± 0,26	2,50a ± 0,16	2,90a ± 0,31	3,16a ± 0,42	2,88a ± 0,28	3,05a ± 0,27	3,14a ± 0,26	3,22a ± 0,46	2,92a ± 0,25	3,2ab ± 0,16	2,83a ± 0,22	2,85a ± 0,28	3,00a ± 0,29
AMZ 0.05mg/kg	7	3,18 ± 0,26	4,03 ab ± 0,51	4,43ab ± 0,54	4,81ab ± 0,44	5,67b ± 0,50	6,21b ± 0,70	4,58ab ± 0,61	3,32a ± 0,50	3,92ab ± 0,57	3,26ab ± 0,32	3,41a ± 0,29	3,02a ± 0,29	3,40a ± 0,25
AMZ 0.10mg/kg	7	3,18 ± 0,26	5,19bc ± 0,89	5,62bc ± 0,23	6,34bc ± 0,41	6,66b ± 0,74	7,26b ± 0,96	6,32b ± 0,67	5,21b ± 0,36	4,98b ± 0,74	3,35ab ± 0,32	3,54a ± 0,50	3,55a ± 0,51	3,31a ± 0,49
AMZ 0.15mg/kg	7	3,18 ± 0,26	5,27cd ± 0,52	6,71bc ± 0,66	7,39c ± 0,76	7,20b ± 0,73	6,57b ± 0,63	5,98b ± 0,31	4,95b ± 0,36	4,78b ± 0,43	4,41b ± 0,44	3,06a ± 0,23	3,40a ± 0,37	2,94a ± 0,33
Xylazine 1.0mg/kg	5	3,18 ± 0,26	7,74 d ± 1,07	8,77c ± 0,71	8,61c ± 0,89	4,80ab ± 0,27	3,17a ± 0,61	2,80a ± 0,49	3,43ab ± 0,57	3,29ab ± 0,22	2,66a ± 0,09	2,94a ± 0,07		

Table 3.

LATENCY TO THE SKIN TWITCH REFLEX (SECONDS)

DRUG INJECTION ↴ TIME AFTER DRUG ADMINISTRATION (MINUTES)

grupo	n	0	5	10	15	30	45	60	90	120	150	180	240	300
Control DMF 5ml	7	3,65 ± 0,28	3,65a ± 0,68	3,72a ± 0,68	4,01a ± 0,41	3,85a ± 0,49	3,85a ± 0,47	3,93a ± 0,39	3,81a ± 0,49	3,50a ± 0,42	3,84a ± 0,49	3,50a ± 0,29	3,56a ± 0,30	3,29a ± 0,690
AMZ 0.05mg/kg	7	3,65 ± 0,28	4,41a ± 1,05	4,66a ± 0,64	4,28ab ± 0,63	4,54ab ± 0,36	4,73a ± 0,55	4,12a ± 0,47	3,95a ± 0,21	3,95ab ± 0,80	3,36a ± 0,21	3,63a ± 0,58	3,71a ± 0,38	3,65a ± 0,25
AMZ 0.10mg/kg	7	3,65 ± 0,28	4,62a ± 0,46	5,05a ± 0,35	5,08ab ± 0,31	5,68ab ± 0,87	5,13ab ± 0,38	4,93ab ± 0,74	4,75ab ± 0,47	4,63ab ± 0,29	3,79ab ± 0,35	3,04a ± 0,49	3,74a ± 0,26	3,20a ± 0,62
AMZ 0.15mg/kg	7	3,65 ± 0,28	4,21a ± 0,28	5,04 a ± 0,34	5,77b ± 0,10	6,18b ± 0,11	6,48b ± 0,33	6,49b ± 0,33	6,32b ± 0,16	5,68b ± 0,33	4,84b ± 0,20	3,97a ± 0,31	3,70a ± 0,29	3,94a ± 0,33
Xylazine 1.0mg/kg	5	3,65 ± 0,28	4,87a ± 0,62	5,26a ± 0,83	4,02ab ± 0,27	4,15ab ± 0,32	3,78a ± 0,21	3,42a ± 0,49	3,97a ± 0,44	3,70a ± 0,31				

Figure legends

Figure 1. Head ptosis in horses after intravenous administration of amitraz at the doses of 0.05, 0.10 and 0.15 mg/kg.

Figure 2. Dose-response curve for the sedative effect of amitraz observed as the head ptosis in horses.

Figure 3. Hoof withdrawal reflex latency (HWRL) in horses after intravenous administration of amitraz at the doses of 0.05, 0.10 and 0.15 mg/kg, or of xylazine at the dose of 1.0 mg/kg.

Figure 4. Dose-response curve for the antinociceptive effect of amitraz observed as the increase in the hoof withdrawal reflex latency (HWRL) in horses.

Figure 5. Skin twitch reflex latency (STRL) in horses after intravenous administration of amitraz at the doses of 0.04, 0.10 and 0.15 mg/kg, or of xylazine at the dose of 1.0 mg/kg.

Figure 6. Dose-response curve for the antinociceptive effect of amitraz observed by the increase in the skin twitch reflex latency (STRL) in horses.

Table legends

Table 1. Head ptosis in horses injected with amitraz (0.05, 0.10 and 0.15 mg/kg). The results are reported as means ± SEM. Means followed by equal letters do not differ from one another. $P<0.05$ (Tukey test).

Table 2. Latency to the hoof withdrawal reflex in horses injected with dimethylformamide (5 mL, control), amitraz (0.05, 0.10 and 0.15 mg/kg) or xylazine (1 mg/kg). The results are reported as means ± SEM. Means followed by equal letters do not differ from one another. $P<0.05$ (Tukey test)

Table 3. Latency to the skin twitch reflex in horses injected with dimethylformamide (5 mL, control), amitraz (0.05, 0.10 and 0.15 mg/kg) or xylazine (1 mg/kg). The results are reported as means ± SEM. Means followed by equal letters do not differ from one another. $P<0.05$ (Tukey test).