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The Sedative, Analgesic and Biochemical Effects of Romifidine in Donkeys

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ABSTRACT

Romifidine was administered intravenously in twelve donkeys in a dose rate of 35, 70 or 100 µg /kg body weight. The levels of sedation and analgesia were graded and recorded.

The sedative effect persisted for, and minutes after intravenous injection of romifidine at 35, 70 and 100 µg/kg b.w. respectively. The degree of sedation was more or less dose dependant and rated from grade 1 to grade 3. The depth of sedation induced by 100 µg/kg was greater than that induced by either 35 or 70 µg/kg .

There was a marked analgesic effect for romifidine. The period of analgesia was shorter than that of sedation. The analgesic effect persisted for , and minutes after intravenous injection of romifidine at 35, 70 and 100 µg /kg b.w. respectively. Intravenous administration of romifidine in a dose rate of 35 and 70 µg /kg induced analgesic effect of grade II. The analgesic effect of romifidine in a dose rate of 100 µg/kg b.w. was excellent (grade 3) as indicated by lack of response to painful external stimulations.

Slight drooping of the head and upper eyelid, increasing of the distance between the ear tips, thickness of the lips, partial prolapse of the penis and frequent urination were all recorded. Significant bradycardia and respiratory depression as well as ataxia were recorded.

It is concluded that romifidine is a potent analgesic and sedative agent in donkeys. Intravenous administration of romifidine at a dose rate of 70 µg/kg produced good sedation and analgesia with mild ataxia and minimal side effects.

INTRODUCTION

Romifidine, imino-imidazolidine derivative, is a relatively recent α2-adrenoceptor agonist (Hall et al, 2001). The other members of this group including xylazine, detomidine and medetomidine have been extensively used in the field of veterinary anesthesiology for their sedative properties (Hall et al, 2001).

Romifidine as well as xylazine and detomidine causing a temporary increase in nociceptive thresholds (Moens et al, 2003). However, these drugs have a dose related effect with an increase in dose resulting in an increase in their degree and duration of action (Freeman and England, 2000).

Romifidine can be included in analgesic and anesthetic protocols to provide additional analgesia in horses (Spadavecchia et al, 2005). Romifidine has a longer period of sedation and analgesia than that of detomidine (Freeman and England, 2000).

Instability and ataxia were less pronounced with romifidine than with detomidine (Hamm et al, 1995). Although all of the alpha 2-agonists exert a marked increase of the uterine pressure, romifidine showed a less uterine pressure duration and strength than that occurred with xylazine and detomidine. However, these differences are not significant (Schatzmann et al, 1994).

Romifidine has been used for sedation of horses, dogs, sheep and goats (England and Clark, 1996; Celly et al, 1997; Amarpal et al, 2002 , Kinjavdekar et al, 2005). However, evaluation of romifidine in donkeys had not been found in the available literature. The purpose of this controlled study is to evaluate objectively the sedative, analgesic and biochemical effects of various doses of romifidine in donkeys.

MATERIALS AND METHODS

Twelve adult healthy donkeys (7 females and 5 males), aged two to seven years and ranged from 100 to 180 kg body weight were used in this study. These animals were kept for a week before experimentation for aclimatization to local conditions. Resting rectal temperture, pulse and respiratory rates were measured and complete blood count was made, before each treatment, to asses animals' health. These animals were randomly divided into three equal groups (4 animals each). Romifidine (Sedivet, Boehringer Ingelheim, France) was administered intravenously in a dose rate of 35, 70 or 100 ug/kg body weight respectively.

Sedation was assesed and graded from 0 to 3 as described by Jochle and Hamm (1986). The chine ground distance (head ptosis, distance from the lower lip to the floor) as well as the distance between the tips of the conchae were measured just before administration of romifidine and every 15 minutes
(from time 0 to recovery). Dropping of the external concheae of the ear and/or upper eyelids, prolapse of the penis and frequency of urination were also observed.

Analgesia was detected and assessed by recording the response of the animal to needle pricks at the same regular intervals. Needle pricks were applied at the shoulder, flank area and perineum. The analgesia was graded from 0 to 3 as described by Jochle and Hamm (1986).

The time of onset, degree, and duration of sedation and analgesia were recorded for 3 hours after drug administration.

Heart and respiratory rates were recorded at 0,15,30,45, 60 min. and at apparent recovery time.

Electrocardiography (ECG):

An electrocardiographic examination was conducted on a donkey injected with 70 µg/ \Kg body weight just before injection, and 30 and 90 minutes post-injection. The echocardiographic traces were obtained using EC 60 cardiac and respiratory monitor, DK-5290, Maralev, Danmark. The base-apex lead system was applied as previously mentioned (Hilwig, 1977 and Ghanem, 1997). Briefly, the right forelimb electrode (RA) was placed along the jugular groove one third of the way up the neck from the torso. The left forelimb electrode (LA) was placed on the ventral midline under the apex of the heart. Both hind limbs (RL and LL) electrodes were attached to the skin over the stifle joints. Alligator clips moisten with alcohol were used.

Biochemical Analysis:

Blood samples were collected at 0,5 ,15 ,30 ,45 , 60 , 90 minutes and 24 hours after administration of romifidine. Serum samples of all animals were separated and used directly for determination of cholinesterase (Den Blowen et al., 1983). Aldolase (Beisenherz, 1953), lactate (Hall et al., 1978) lactate dehydrogenase (Buhl and Jackson, 1978), creatin phosphokinase (Rosano et al, 1976), Aspartate aminotransferase (Belifield and Goldburg, 1971) the serum was also used for estimation of glucose (Borham and Trinder, 1972), insulin hormone according to Dudley (1985), urea (Patton and Crouch, 1977) and creatinine (Henry, 1974).

RESULTS

Sedative Effect:

Intravenous injection of romifidine induced a rapid loss of coordination and apparent sedative effect within 1-3 minutes. The mean of maximum sedation as indicated by minimal distance between the lower lip and the ground was (15 -25 cm), (10-20 cm) (0 - 10 cm) respectively. The maximal dropping of the head was achieved few minutes after intravenous injection of romifidine respectively. The sedative effect was persisted for , and
minutes respectively (Table 1). The degree of sedation was more or less dose dependent and rated from grade 1 to grade3. The depth of sedation induced by 35 ug/kg (grade1) was less than that induced by 70ug/kg (grade 2) or 100 ug/kg (grade 3).

During the period of sedation there was marked drooping of external conchae of the ear, increasing of the distance between the ear tips, thickness of the lips, drooping of the upper eyelids and partial prolapse of the penis.

Increased urination commencing about 70 to 100 minutes after administration of romifidine was observed along this study in all groups.

Analgesic Effect:

The analgesic effect of romifidine was recorded in table1. Injection of romifidine at 35 µg/kg b.w. induced good analgesic effect (Grade II). Meanwhile, the higher doses (70 or100 µg/kg) induced an excellent level of analgesia (Grade III) which indicated by lack of response to painful stimulation .

The period of analgesia was shorter than the period of sedation (table 1). The analgesic effect persisted for 60± , 70± and 75± minutes following i.v. injection of romifidine at a dose rate of 35, 70, 100 µg/kg respectively. Most of these animals, even in deeply sedated ones, were able to raise their hind limbs and to kick when painful stimulation was applied.

In all of the examined groups the degree of analgesia was more pronounced at the level of the head, neck, shoulder, and thoracic and abdominal walls. The degree of analgesia was less pronounced at the perineum and the hind limbs.

Ataxia was variable from mild to moderate in animals treated with romifidine. While transient and mild ataxia were associated with lower doses (35 and 70 µg/kg), moderate ataxia recorded mostly at the higher dose (100 µg/kg). None of the treated donkeys of the three groups along this study showed recumbency.

Bradycardia was also observed in all animals which received romifidine (Table 2). Heart rates were significantly reduced 5 minutes after i.v. injection of romifidine in all groups where the means of heart rate were 36.4 + 2.6, 33.5+2.5 and 28.3+- 3.2 in the three groups respectively. Twenty five

beats/minute was the lowest heart rate recorded. Auscultation showed also irregular rhythm and drooped beats. The heart rates returned to up to its normal levels after 60 to 90 minutes of induction of sedation.

Intravenous injection of romifidine in a dose rate of 35 µg/kg body weight showed a slight decrease in the respiratory rate which extended up to the end of observation period (Table 3). Intravenous injection of romifidine in a dose rate of 70 or 100 µg/kg body weight showed a significant decrease which also extended up to the end of observation period. The respiratory rate didn't return to the normal level in all of the three examined doses.

Increased urination was frequently observed in all of the three groups in this study. The time from intravenous injection of romifidine to first urination ranged between 70 to 100 minutes. Animals received romifidine showed no signs of sweating or piloerection. Moreover, recumbency did not occur even in deeply sedated animals (100 uk/kg) but protrosion of the penis was observed in some animals.

Effect of Romifidine on ECG Findings

ECG findings of Romifidine-injected donkey showed a marked reduction in the heart rate (bradycardia) denoted by prolongation of the R-R interval (period between 2 successive R waves). The myocardial contraction is markedly reduced as denoted by reduction in the amplitude of R wave (30 minutes after romifidine injection). The ventricles start to regain their contraction 75 minutes after romifidine injection although the heart rate was not regained. Ninety minutes after romifidine injection, the ventricular contraction increases but the bradycardia persists (Figures 1A-1D)

Biochemical Analysis:

Result of biochemical analysis (Tables 4-7) revealed a significant increase in serum glucose level that extended from 30 to 90 minutes. This increase become non significant at 24 hr from injection of Romifidine in GI and remain significant till 24 hr in GII and GIII. I

Insulin hormone showed significant decrease that extended from 15 to 90 min in GI and GII, this decrease become non significant at 24 hr, when compared with the control level.There was non significant increase in the levels of serum urea and creatinine in GI and GII, while GIII revealed a significant increase in serum urea nitrogen level were only recorded at 60 and 90 min.

A significant decrease in Ach E was recorded at 30 to 90 min in GI of Romifidine injected donkeys. This decrease become non significant at 24 hr. While in GII and GIII a high significant decrease were recorded extend from 15 min to 24 hr from injection. Also, significant decrease in aldolase activity extended from 30 to 60 min. This decrease become non significant at 24 h in GI and GII while extended to 24 h in GIII. Serum lactate level showed significant increase that extended from 30 to 90 min. in GI. This increase remain significant till 24 hr in GII and GIII of Romifidine administered donkeys compared with the control level.

Marked increase in serum LDH, CPK and AST levels were recorded in donkeys injected with 35 µg/kg romifidine, while a highly significant increase in LDH was recorded at 45 and 60 min in in donkeys injected with 70 and 100 uk/kg romifidine. A significant increase of LDA levels at 90 min. was recorded, this increase become non significant at 24 hr from injection in GII and remain significant till 24 hr in GIII. Also, CPK, AST showed significant increase extended from 30 to 90 min. This increase become non-significant at 24 hr from injection in GII and remain significant till 24 hr for injection in group 3.

Discussion

Romifidine is one of the relatively new agents of α2-adrenoceptor agonist. This study demonstrated the potent sedative and analgesic effects of Romifidine in donkeys. The onset of sedation started soon after intravenous injection of romifidine (1-3 minutes). There was no difference of latency in animals injected with the three doses of romifidine.

The result of this study proved that the degree of sedation and analgesia of romifidine in donkeys was dose dependant. A positive correlation between its doses and degrees of sedation and analgesia was recorded.

Sedative effect of romifidine in horses is similar to xylazine and detomidine but is longer lasting and produces less ataxia (England et al, 1992). The result of this study showed that the grade of sedation was dose dependent and rated from grade 1 to grade3. The depth of sedation induced by 35 µg kg (grade1) was less than that induced by 70 µg /kg (grade 2) or 100 µg /kg (grade 3). This result agrees with previous reports in horses (England et al, 1992, Freeman and England 2000 and Freeman et al, 2002) and disagrees with others (Hamm et al, 1995). The later authors reported that a dose of 120 µg /kg body weight romifidine was only equal to, or even less effective than a dose 80 µg/kg body weight.

Head height, distance between the ear tips, thickness of the lips, drooping of the upper eyelids and partial prolapse of the penis were all recorded in this study as signs of muscle relaxation that is

associated with romifidine. These findings are in consistent with that recorded in horses (Freeman and England, 1999 and Freeman and England, 2000).The observed thickening of the lip is attributed to the development of head edema that is associated with drooping of the head .

There was a marked analgesia in the three tested doses of romifidine in donkeys. The degree and duration of analgesia was more or less dose dependant. The durations of analgesia were between 60± , 70± and 75 ± in the three groups respectively. This result disagrees with another report (Hamm et al, 1995) who stated that romifidine unexpectedly has no analgesic effects at any time in horses. However, our result agrees with other reports who stated that romifidine can be included in analgesic and anesthetic protocols to provide additional analgesia in horses (Spadavechia et al, 2005). Moreover, the analgesic effects of romifidine were similar to that produced by detomidine that characterized by prolonged and intense analgesia (England and Clarke, 1996).

Marked levels of ataxia were observed with romifidine in this study especially in the higher dose (100µg/kg). The degree of ataxia seems to be dose dependant. This result might agree with that described by Browning and Collins (1994). The used doses of romifidine didn't lead to recumbency or falling down of any of the animals of this study. This result might disagree with that reported in horses (Freeman and England, 2000) who recorded recumbency with romifidine in some cases. However, romifidine produces less severe ataxia than that recorded with detomidine (Englandet al, 1992, Browning and Collins , 1994,Hamm et al, 1995 and Freeman and England 2000).

The increased frequency of urination associated with romifidine is possibly through inhibition of antiduretic hormone (ADH) release. Using high doses of alpha 2 adrenoceptor agonists is usually associated with diuresis that possibly assisted by hyperglycaemia (Short,1992 , Freeman and England, 2000, and Hall et all, 2001). The increase of urine production over 90 minutes is accompanied by an increase in glucose excretion while creatinine clearance remains constant (England and Clarke, 1996; Gasthuys et al, 1996 and Hall et all, 2001).

A substantial cardiovascular changes specially bradycardia had been observed after administration of Romifidine. Similar findings had been reported in horses (England and Clarke, 1996 and Freeman et al, 2002). A significant bradycardia was recorded 5 minutes after administration of romifidine in all groups. The bradycardia was significant even in the low dose (35 µg /kg). The cardiovascular depression including bradycardia and initial hypertension followed by hypotension was dose dependant in horses (Freeman et all, 2002). Intravenous injection of romifidine showed a decrease in the respiratory rate which extended up to the end of observation period in all groups. Similar respiratory depression had been reported in horses (Freeman et al, 2000).

ECG findings showed that romifidine injection induced marked bradycardia in donkeys indicated by prolongation of the R-R interval. Previous researches showed that romifidine reduced the heart rate in dogs in a dose-dependent manner (Lemke 1999). Electrocardiographic examination of a donkey from the second group was conducted to investigate whether romifidine produce changes in heart contraction similar to those of horses. ECG findings showed that romifidine injection induced bradycardia in donkeys indicated by prolongation of the R-R interval. Previous researches showed that romifidine reduced the heart rate in a dose-dependent manner in dogs (Lemke 1999) and in horses (Gasthuys et al, 1990). Consistently with this finding, Figueiredo et al (2005) noticed a reduction of the heart rate following IV injection of romifidine with doses of 40 and 120 µg/kg. As a member of Alpha2 adrenoceptor agonists, romifidine reduces the heart rate via vagally-mediated reflex bradycardia and partly from central sympathetic depression (England and Clarke,1996).

The ECG tracing showed also a reduction in the R wave amplitude which indicates suppression of the myocardial contraction during systole, and hence the cardiac output. This finding is consistent with those demonstrated by Melissa (2003).

Further studies are required to completely investigate the effect of romifidine on the ECG finding in donkeys.

The result of this study proved that romifidine is a potent sedative and analgesic drug in donkeys. Although the recommended dose of romifidine in horses ranged between 40 to 120 µg/kg body weight, the result of this study showed that the recommended intravenous dose of romifidine in donkeys is 70 µg/kg. This dose was associated with marked degree of sedation and analgesia with minimal side effects.

The transient increase in glucose level within 30-90 min. following the low Romifidine dose is a recognized effect of α_2 agonist that was associated with growth hormone stimulation and insulin suppression through direct inhibitory effect of Romifidine on β cell of pancrease (Dollery, 1991). Similar results were obtained in female goats by Aithal et al. (2001) and Amerpal et al. (2002) and in horses by Gsthuys et al. (1996), who indicated that α_2 adrenoreceptor agonist lowered plasma glucose ($P < 0.01$),

They added that the changes in plasma insulin and glucose differ significantly between doses as α_2 agonist increased incidence of is let cell hyperplasia in the pancrease which was attributed to the observed hyperglycemic action of the compound rather than to the toxic effect Lyons et al. (1997). In addition increase in the catabolic hormone such as cortisol that may be released in response to stress and alter the fuction of insulin hormone leading to hyperglycemia (Short, 1992). Moreover stimulation of α_2 adrenorceptor leads to inhibition of beta cell electrical activity and suppress insulin secretion (Bokvist et al., 1991) as well as administration of sedative caused protracted decrease in basal, local cerebral metabolic rate for glucose utilization (Knapp et al., 2002).This increase become non significant at 24 hr although creatinine showed non significant increase through the whole time of experiment.

There were non significant changes in serum urea and creatinine level at 24 hr following intravenous injection of romifidine in a dose rate of 35 or 70 ug/kg body weight. Similar observations were reported in buffalo and goats (Amarpale et al. , 2002 and Sharma et al. (2004). The recorded transient significant decrease in Ach E in GI of Romifidine administried donkeys may be explained as α_2 agonist reduce the amplitude of electrical activity in the brain and shift frequency responses confiriming the clinically observed depression (Stenberg et al., 1986) as well as reduction in cerebral blood flow that has been recorded in α_2 adrenorceptor agonist treated horses since the head down position could results in vascular congestion and potentially cerebral edema. In addition analgesic and hepnotic may causes changes in brain serotonin (Singh and Sanyal, 2003).

Regarding the highly significant decrease in Ach E that extend to 24 hr after injection of high dose of Romifidine. It may be attributed to damage in brain tissue (Yong, 1989) as phsychomotor excitement, hyperactivity and irreversible brain damage based on abnormal changes in neuronal system (Kamei et al., 2003).

These results were confirmed by MacDonald and Virtamen (1992) who showed that Romifidine inhibit nor adrenaline release from sympathetic nerve terminals and directly increase the release of acetyl choline from parasympathetic nerve in the heart. Thus the symptoms seen with high doses of Romifidine comprised tremors ataxia and convulsion indicate the marked toxic effect of Romifidine at high dose.

CPK is higher in skeletal muscle, heart tissue and brain. It is elevated in disease of myocardium and nervous tissues (Hassanien et al., 2003).Romifidine administration in donkeys induced significant increase in CPK, LDH and GOT during the first 90 min. of administration of 70 ug/kg. These changes extend to reach 24 hr in GIII when compared to control. This result may be explained as Romifidine produced marked changes in cardiovascular function of healthy horses as α_2 agonist significantly decrease the heart rate. This decrease may be in the form of bradycardia and atrioventricular block may be observed Raekallio et al. (1990) leading to ischemia that indicated by cellular injury of the myo cardium with a prominent biochemical and ultra structural changes in key subcellular organells, Hodgson and Levi (1995) that changes were similar to those produced by cardio toxicity of catecholamines. Thus Romifidine at high dose caused damage to heart tissue that mediate leakage of LDH from cells and escape of the enzyme into the blood leading to decrease in cell viability (Young, 1989).

Regarding AST our results were came in accordance with Abeir (1999) who reported that analgesic may cause liver dysfunction confirmed in our results by the significant increase in serum urea.

Moreover the significant increase in serum CPK and AST could be related to cerebral and cardio vasculare disorder which leads to decrease in cell viability and cellular damage leading to escape of these enzymes into blood (Abdel-Aleim et al., 2005).

Romifidine induce significant reduction in respiratory rate via the direct depressive effect of the drug on the respiratory center (Sharma et al., 2004). So arterial oxygen level drop during the more profound effect of α_2 agonist as increasing concentration of α_2 agonist resulted in alteration in ventilation and marked lowered in oxygen saturation and arterial oxygen level (Vaha et al., 1991). Thus in the absence of oxygen the body depends on glycolysis for it's energy requirement with marked production of lactate from pyruvate by the help of lactate dehydrogenase enzyme (Bakry, 1994).

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Table 1: The Sedative and Analgesic Effects of Romifidine in Donkeys.

Drug / Dose	Sedation		Analgesia	
	Grade	Duration	Grade	Duration
Romifidine 35 ug/kg	I	60- 70 minutes	1I	30 -45 minutes
Romifidine 70 ug/kg	II	65-75 minutes	III	45- 70 minutes
Romifidine 100 ug/kg	III	90- 100 minutes	III	60- 90 minutes

Table 2: Mean+ SD of the heart rates in donkeys injected intravenously with different doses of Romifidine.

Dose	Heart Rate (beats / minute) Time (minutes)							
	0	**5**	**15**	**30**	**45**	**60**	**90**	**Recovery**
35 µg / kg	46.9±1.9	36.4 ± 2.6	37.2 ± 1.8	38.5 ± 1.3	40.7 ±1.1	42.1 ± 1.6	45.1 ± 2.4	46.3 ± 3.1
70 µg / kg	45.7 ± 2.1	33.5 ± 2.5	34.3 ± 2.2	35.8 ± 2	37.9 ± 1.7	39.3 ± 2.1	45.8 ± 4.2	46.3 ± 3.5
100 µg / kg	39.5 ± 1	28.3 ± 3.2	27.3 ±1.5			40 ± 5.4	44.8 ± 5.7	44.8 ± 4.9

Table 3: Mean+ SD of the respiratory rates in donkeys injected intravenously with different doses of Romifidine.

Dose	Respiratory Rates (breath/ minute) Time (minutes)							
	0	**5**	**15**	**30**	**45**	**60**	**90**	**Recovery**
35 µg / kg	16.3 ± 1.1	12.6±1.9	12.8±1.8	12 ±1.6	12±2.1	11.2±1.7	9.9±1.2	8.6±1.4
70 µg / kg	17±2.3	10.8±1.7	11±1.2	11±0.8	9.5±1.3	8.7±1.1	7.7±0.8	7.3±0.7
100 µg / kg	14.3±1.3	13±2.4	12.7±1.9	10.4±1.1	9.2±1.8	8.7±1.4	6.7±1.7	6.5±1.1

Table 4: Mean values of serum (glucose and insulin) level of male donkeys through 24 hour after injection of different concentration of Romifidine.

Group	Parameter	Time							
		Control	5 min	15 min	30 min	45 min	60 min	90 min	24 hr
GI 0.40 ml/100 kg b.w.	Glucose (mg/dL)	96.00 ±5.78	103.11 ±5.42	102.31 ±5.36	143.15** ±7.53	141.00** ±7.43	130.10* ±6.84	125.22* ±6.99	111.03 ±5.85
	Insulin (µIU/ml)	22.60 ±1.60	17.30 ±1.59	16.91* ±1.18	11.91** ±0.92	14.90** ±1.03	14.75* ±0.99	17.00* ±1.18	19.51 ±1.02
GII 0.80 ml/100 kg b.w.	Glucose (mg/dL)	91.20 ±4.78	92.60 ±4.87	129.10* ±6.79	189.15** ±9.85	148.13** ±7.97	143.6** ±7.55	126.22** 6.59	125.00* ±6.36
	Insulin (µIU/ml)	22.99 ±1.18	18.97* ±0.99	18.71* ±0.98	10.31** ±0.84	15.21** ±0.89	15.10** ±0.79	19.01* ±0.95	23.11 ±1.21
GIII 1.20 ml/100 kg b.w.	Glucose (mg/dL)	93.11 ±4.95	10.90 ±6.92	205.03** ±10.79	203.00** 10.68	163.91** 8.87	150.01** ±7.89	132.10** ±6.95	121.50* ±6.22
	Insulin (µIU/ml)	21.81 ±1.16	18.19* ±0.95	11.36** ±0.67	11.50** ±0.86	16.53** ±0.87	16.23** ±0.93	16.10** ±0.95	15.31** ±0.80

*** Significant at (P<0.05).**
**** Highly significant at (P<0.01).**

Table (5): Mean values of serum (urea and creatinine) level of male donkeys through 24 hour after injection of different concentration of Romifidine.

Group	Parameter	Time							
		Control	5 min	15 min	30 min	45 min	60 min	90 min	24 hr
GI 0.40 ml/100 kg b.w.	Urea	24.61 ±2.45	27.71 ±2.66	29.91 ±2.57	29.71 ±2.06	31.61 ±2.99	30.43 ±3.01	31.15 ±2.63	26.20 ±2.36
	Creatinine	1.15 ±0.08	1.19 ±0.06	1.12 ±0.10	1.40 ±0.13	1.44 ±0.17	1.39 ±0.12	1.20 ±0.11	1.16 ±0.12
GII 0.80 ml/100 kg b.w.	Urea	23.56 ±2.86	27.18 ±2.51	26.37 ±2.45	29.94 ±2.61	30.61 ±2.88	34.30 ±3.70	35.12 ±3.25	30.15 ±2.58
	Creatinine	1.10 ±0.09	1.12 ±0.08	1.23 ±0.10	1.41 ±0.11	1.45 ±0.16	1.58 ±0.17	1.36 ±0.11	1.07 ±0.08
GIII 1.20 ml/100 kg b.w.	Urea	25.11 ±2.54	29.50 ±2.70	32.19 ±2.74	33.61 ±2.94	35.16 ±3.53	40.21* ±3.11	39.10* ±3.30	32.15 ±2.69
	Creatinine	1.12 ±0.10	1.15 ±0.08	1.22 ±0.09	1.51 ±0.16	1.67 ±0.20	1.52 ±0.10	1.39 0.08	1.18 ±0.07

*** Significant at (P<0.05).**

Table (6): Mean values of serum (Aldolase, Ach E and Lactate) of male donkeys through 24 hour after injection of different concentration of Romifidine.

Group	Parameter	Time							
		Control	5 min	15 min	30 min	45 min	60 min	90 min	24 hr
GI 0.40 ml/100 kg b.w.	Aldolase (µ/L)	6.90 ±0.38	6.30 ±0.36	5.59 ±0.29	5.35* ±0.28	4.18** ±0.22	4.97* ±0.26	5.72 ±0.30	6.85 ±0.39
	Ach E	13.92 ±1.04	13.22 ±0.93	11.60 ±0.91	9.72* ±0.81	8.11** ±0.72	8.90* ±0.61	9.33* 0.79	10.69 ±0.95
	Lactate (µ/L)	18.50 ±1.90	19.91 ±1.04	23.11 ±2.74	34.6* ±2.78	47.00** ±3.52	51.13** ±4.78	30.44* ±2.33	27.10 ±2.26
GII 0.80 ml/100 kg b.w.	Aldolase (µ/L)	7.19 ±0.38	7.01 ±0.36	5.99 ±0.31	4.15** ±0.21	4.03** ±0.21	3.97** ±0.22	5.80* ±0.30	6.66 ±0.39
	Ach E	14.10 ±0.94	11.51 ±0.69	9.95* ±0.58	6.10** ±0.41	5.96** ±0.42	6.00** ±0.49	6.99** 0.52	10.11* ±0.63
	Lactate (µ/L)	17.69 ±1.22	23.00 ±2.10	32.90* ±3.01	46.50** ±3.50	65.05** ±4.55	68.00** ±5.21	63.11** ±5.42	40.10** ±3.63
GIII 1.20 ml/100 kg b.w.	Aldolase (µ/L)	7.33 ±0.42	5.99 ±0.33	5.21* ±0.27	4.66** ±0.25	4.21** ±0.28	4.26** ±0.22	4.59** ±0.31	5.94* ±0.27
	Ach E	14.29 ±0.94	12.11 ±0.73	7.99** ±0.59	8.11** ±0.52	5.21** ±0.42	6.82** ±0.55	5.19** ±0.46	7.11** ±0.47
	Lactate (µ/L)	17.85 ±0.92	19.11 ±1.05	35.00** ±2.40	51.81** ±4.26	67.18** ±4.15	56.33** ±2.43	71.60** ±5.87	39.00** ±2.05

*** Significant at (P<0.05).**

**** Highly significant at (P<0.01).**

Table (7): Mean values of serum (LDH, CPK and Got) of male donkeys through 24 hour after injection of different concentration of Romifidine.

Group	Parameter	Time							
		Control	5 min	15 min	30 min	45 min	60 min	90 min	24 hr
GI 0.40 ml/100 kg b.w.	LDH (µ/L)	401.00 ±21.10	451.00 ±23.73	473.00 ±24.89	495.00 ±31.31	463.00 ±29.63	453.00 ±23.84	445.00 ±23.42	440.00 ±23.15
	CPK (µ/L)	9.20 ±0.58	8.91 ±0.46	10.90 ±0.77	11.31 ±0.79	11.90 ±0.98	11.53 ±0.85	9.66 ±0.53	8.90 ±0.48
	GOT (µ/L)	44.18 ±3.26	43.90 3.62	49.00 ±3.57	53.00 ±3.78	59.00 ±4.54	49.03 ±3.57	49.17 ±3.57	49.00 ±3.80
GII 0.80 ml/100 kg b.w.	LDH (µ/L)	403.00 ±23.89	416.00 ±21.84	463.00 ±24.36	472.00 ±24.84	586.00** ±30.84	593.00** ±31.21	520.00* ±27.36	509.00 ±32.10
	CPK (µ/L)	9.51 ±0.84	9.10 ±0.47	10.19 ±0.57	14.25* ±0.99	16.33* ±1.39	13.91* ±0.73	13.10* ±0.69	11.91 ±0.63
	GOT (µ/L)	42.15 2.26	44.10 ±2.68	51.18 ±2.79	52.90* ±2.89	69.11** ±3.63	54.10* 2.84	51.89* ±2.31	44.13 ±2.36
GIII 1.20 ml/100 kg b.w.	LDH (µ/L)	395.00 ±22.7	466.00 ±24.52	503.00* ±26.47	581.00* ±30.57	661.00** ±34.78	670.00** ±35.29	511.00* ±26.89	493.00* ±25.94
	CPK (µ/L)	10.20 ±0.96	11.61 ±81	16.31* ±0.99	19.51** ±1.21	20.15** ±1.50	18.00** ±1.15	17.33* ±1.11	13.94* ±0.93
	GOT (µ/L)	41.01 ±2.26	49.00 ±2.57	54.9* ±2.84	63.60** ±3.31	69.21** ±3.63	59.00** ±2.63	53.90* ±2.63	51.00* 2.68

*** Significant at (P<0.05).**

**** Highly significant at (P<0.01).**

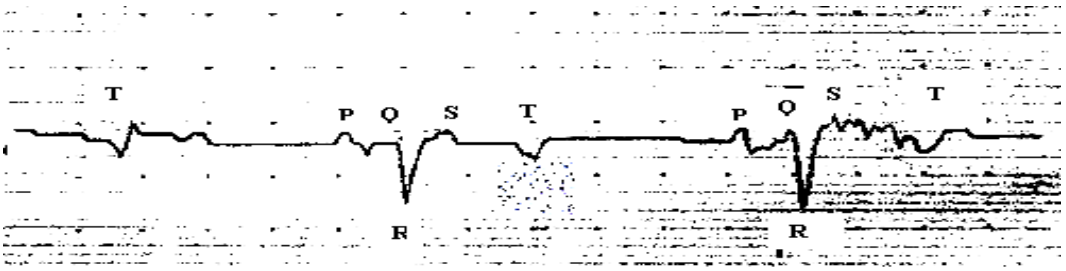
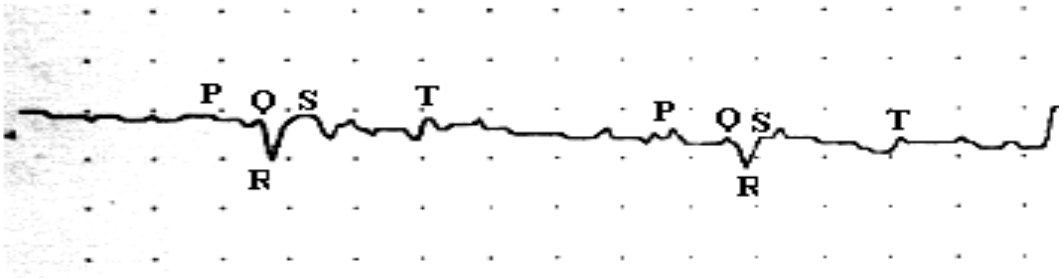


Fig 1-A. ECG trace from a donkey before injection of romifidine. P wave indicate atrial contraction. QRS complex denote ventricular contraction. The biphasic T-wave indicates ventricular relaxation. Notice that the period between the 2 successive R wave is 6 boxes. The heart rate is 35 /min.



Fiq 1B.ECG trace from a donkey 30 minutes after injection of romifidine. Notice the short amplitude of R wave that denotes reduction in myocardial contraction of ventricles. The period between the 2 successive R wave (R-R interval) is increased (8 boxes) indicating slow heart beats (bradycardia) compared to control (before injection).

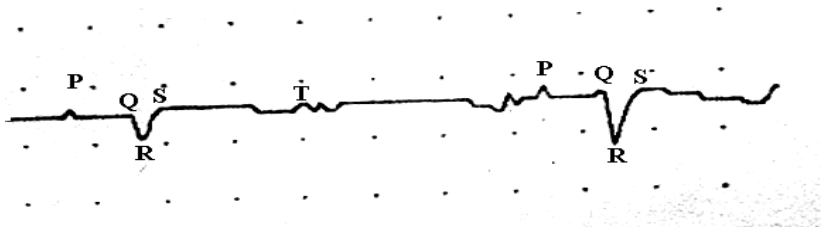


Fig1 C. ECG trace from a donkey 75 minutes after injection of romifidine. Notice that the gradual increase in the R-wave amplitude that denote regaining myocardial contraction.

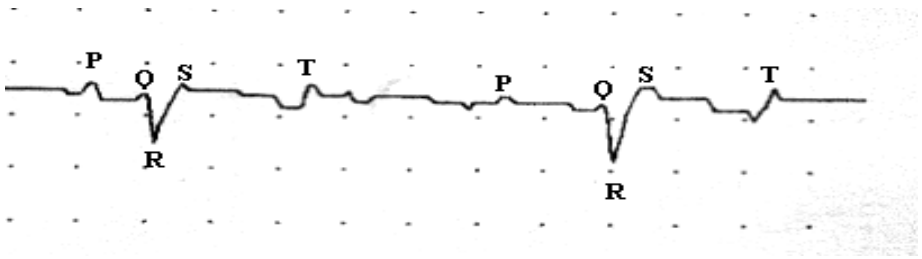


Fig 1D- ECG trace from a donkey 90 minutes after injection of romifidine. Notice the gradual increase in the R-wave amplitude that denotes regaining myocardial contraction. The bradycardia still present because the R-R interval still longer.