Evaluation of different central nervous system depressors combined with ketamine for anaesthesia in mice

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ABSTRACT: The aim of this study was to compare some depressors of the central nervous system combined with ketamine in order to find an adequate scombination for anaesthesia in mice, coupled with a simple, easy to use and reliable method. Forty Swiss OF-1 mice (*Mus musculus*), 20 females and 20 males with a body weight from 35 to 45 g aged from 12 to 16 weeks, were used to evaluate one of the following central nervous system depressors (CNSD): acepromazine (5 mg/kg), diazepam (5 mg/kg), medetomidine (1 mg/kg), midazolam (5 mg/kg) and xylazine (10 mg/kg) combined with the dissociative anaesthetic ketamine (100 mg/kg) by the intraperitoneal route. Different parameters were evaluated at regular intervals to assess the depth of anaesthesia (time of induction, time of loss and recovery of pedal withdrawal reflex, time of recovery from the anaesthesia), and respiratory and heart rate and oxygen saturation. Most of the assessment times and physiological parameters were exhibited earlier in females than in males but, in most cases, these differences were not significant. The diazepam combination resulted in death in half of the male group. Significant differences for the combination comparison were found for induction, pedal withdrawal reflex and recovery from anaesthesia, as well as for respiratory and heart rate and oxygen saturation. The best results for mice of both genders, i.e. induction, maintenance and recovery from anaesthesia were more stable with α_2-agonists than with other combinations (benzodiazepines or acepromazine), which did not reach a good anaesthetic level, that is, an adequate anaesthetic plane with an absence of the pedal withdrawal reflex and the maintenance of stable vital constants.

Keywords: anaesthesia; acepromazine; diazepam; midazolam; medetomidine; xylazine

Rodents have become frequent patients in veterinary clinics. These animals present several pathologies which have to be diagnosed and treated by clinicians (Crowell-Davis and Murray, 2006). Veterinary researchers must ensure their welfare and prevent unnecessary pain and suffering to them.

The difficulty of anaesthetic procedures in small rodents, especially mice, lies in their small size (Flecknell 2009), i.e., for venous access or intubation (Henke et al. 2004). Injection, rather than inhalation of anaesthesia could be a method of choice in mice (mainly by subcutaneous or intraperitoneal routes, *s.c.* or *i.p.)* due to it being easier and, also, not requiring costly apparatus, since not all centres (laboratories and/or small clinics) possess these resources for performing inhalatory anaesthesia, specifically in mice. Most of the disadvantages associated with inhalants involve the technical skills required and the expense of special equipment (Murray et al. 2000).

Well controlled anaesthesia is the basis for minimising the potential for intra-operative complications and for ensuring the reproducible success of surgical operations. Veterinary investigations are the basis for a proper anaesthetic protocol that could be established to ensure safe anaesthesia. In order to carry out experimental procedures and/or specimen taking, or making different diagnosis tests, on many occasions the mice require some form of physical or chemical restraint (Cruz et al. 1998). Injectable (usually *i.p.*) anaesthesia is a method of choice in these small rodents (Arras et

al. 2001), using drugs for parenteral use, such as phenothiazines, benzodiazepines, and α_2-agonists combined with an anaesthetic substance such as a dissociative anaesthetic (ketamine), that cannot fulfil by itself all the requirements of anaesthesia (e.g., analgesia, hypnosis, muscular relaxation).

The goal of this study was to compare each of the central nervous system depressors combined with the dissociative anaesthetic ketamine in order to determine a good anaesthetic combination by the intraperitoneal route in Swiss OF-1 mice (*Mus musculus*).

MATERIAL AND METHODS

Animals. Forty outbred Swiss OF-1 mice (*Mus musculus*), twenty males and twenty females from the Central Service of Animal Experimentation at the University of Cordoba (Spain), with a body weight from 35 to 45 g and ages ranging from 12 to 16 weeks were used. Males were randomly divided into three groups, two groups of seven animals and one group of six animals. Each group was housed in plastic cages at 20 °C to 24 °C, 45% to 65% relative humidity and with a 12 h light/dark cycle with "*ad libitum*" access to drinking water and a standard food pellet diet (Panlab®, Barcelona, Spain). Each group of males was injected with two different protocols. The first group (n = 7) was injected with xylazine/ketamine, and, one week later, with medetomidine/ketamine. The second group (n = 7) was injected with midazolam/ketamine and one week later with ketamine alone for reference. The third group (n = 6) was injected with acepromazine/ketamine, and, one week later, with diazepam/ketamine. The same treatment was carried out for the females (Table 1).

The research procedures were carried out after approval by the animal care committee of the University of Cordoba (Spain) and in concordance with the European Directive for the Protection of Experimental Animals (Directive 2010/63/UE).

Table 1. Distribution of the study groups

	1 (n = 7)	2 (n = 7)	3 (n = 6)
Females/Males	X/K	Mid/K	A/K
		*	
	M/K	K	D/K

X/K = xylazine/ketamine, Mid/K = midazolam/ketamine, A/K = acepromazine/ketamine, M/K = medetomidine/ketamine, K = ketamine, D/K = diazepam/ketamine
*one week later

Drug procedure. Acepromazine (Calmo Neosan®, Pfizer, Madrid, Spain), diazepam (Valium®, Roche, Madrid, Spain), medetomidine (Domtor®, Pfizer, Madrid, Spain), midazolam (Dormicum®, Roche, Madrid, Spain) or xylazine (Rompum®, Bayer, Barcelona, Spain) were used combined with ketamine (Imalgene 1000®, Merial, Lyon, France), as anaesthetic drugs.

Drugs were prepared as a mixture of one of the central nervous system depressors (CNSD): acepromazine (5 mg/kg b.w.), diazepam (5 mg/kg b.w.), medetomidine (1 mg/kg b.w.), midazolam (5 mg/kg b.w.) or xylazine (10 mg/kg b.w.), and also saline (0.9% NaCl) solution (Saline Physiology Braun®, Barcelona, Spain), with a dissociative anaesthetic: ketamine (100 mg/kg b.w.). All anaesthetic combinations were diluted with saline to 6 ml volume. From these preparations, a volume dose of 6 µl/g (total volume ranging between 0.21–0.27 ml) was administered as a single injection by the intraperitoneal route using an insulin needle-syringe pack (40 IU or 1 ml). Atipamezole (Antisedan®, Pfizer-Madrid, Spain), at 1 mg/kg b.w. dose was used for reversing after 90 min of ketamine + medetomidine injection; if it medetomidine is not antagonized, its effects become greatly prolonged in time and thus its reversal has been recommended (Plumb 2008; Baker et al. 2011) with atipamezol (α-2-antagonist). We have established 90 min as the time of administration, since rarely will an experimental process in mice require a longer period.

Experimental procedure. One group of animals (n = 6/7) was evaluated daily with a single anaesthetic protocol, beginning randomly with females or males in each protocol.

Mice were weighed (Sartorius BP210 D electronic balance) and their tails marked with an indelible pen. An appropriate volume of the drug mixture was injected by the *i.p.* route, lateral to the mid line next to the umbilicus, as a single dose.

After injection, each mouse was placed in a cage alone and transferred to a room in which an observer-researcher assessed time-related anaesthesia parameters. The observer-researcher did not know what combination had been injected. To exclude any circadian rhythm and environmental effects, the experiments were always performed during the daytime between 9.00 and 12.00 a.m., and under the same environment-controlled conditions mentioned above.

Time-related anaesthesia parameters. After administration (recorded as injection time), the animal, in an individual cage, was observed for incoordination and ataxia (recorded as "ataxia time") until it lost its righting reflex (recorded as "hypnosis time"). Then it was laid in dorsal recumbency without any fixation on a homeothermic blanket to maintain its body temperature. The animals breathed room air for the duration of the procedure. The depth of the anaesthesia was evaluated by assessing the pedal withdrawal reflex every five minutes (the legs of the animal were, alternately -right and left limbs-slightly extended and the metacarpal region of the hind foot was pinched between the index finger and the thumb) (Alves et al. 2007). The assessment of the anaesthetic parameters was always carried out by the same researcher.

Indicators for anaesthesia depth. A series of simple tests were carried out on each mouse to evaluate the intensity and duration of the anaesthesia. The indicators were adapted from published protocols (Smith 1993; Flecknell 2009). To reduce sources of variation in response to the stimuli, all tests were conducted and assessed by the same operator. The intensity of the anaesthesia was assessed by recording the presence or absence of the pedal reflex as mentioned above. The tests related to the duration of the anaesthesia included the recovery time (the animal has recovered its pedal reflex but remains in recumbency), righting (the animal modifies its lateral decubitus position and begins to try to stand), and exploratory phase (the animal starts to walk and sniffs the different areas of the cage)

Physiological measurements. The physiological parameters evaluated included respiratory rate (breaths/min), cardiac rate (beats/min) and blood oxygen saturation (percentage). The respiratory rate (RR) was monitored at 10, 20, 40 and 60 min; the heart rate (HR) and oxygen saturation (Ox) was monitored at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75 and 90 min as from the administration of the anaesthetic combination. All measurements were taken when the mouse was placed in dorsal recumbency on the homeothermic blanket up to the righting reflex recovery. Respiratory rate was assessed (always by the same operator) by evaluating movements of the respiratory muscles during sleep. Heart rate and oxygen saturation were evaluated by a veterinary pulse-oxymeter (2000T veterinary transflectance sensor). The pulse-oxymeter probe was adapted over the base of the tail when the animal was laid in dorsal recumbency.

Data and statistical analysis. Data were recorded in a Microsoft-Excel sheet and shown as mean, number of data (n) and standard deviation (s.d.). Statistical analysis was made using Statgraphics: Chi-Square "χ^2" and Shapiro-Wilks statistic "W" were used for checking the fit of a normal (Gauss) distribution. A non-parametric test (Kruskal-Wallis), when normal distribution data were rejected, was carried out. Non-parametric (Spearman rank) correlations were made. The minimum significant value for a statistical hypothesis was $P < 0.05$.

RESULTS

Time span of different anaesthetic stages and planes

Time-related parameters of anaesthesia for male and female mice are listed in Table 2 in which are recorded mean values, number of measurements (n) and standard deviation (s.d.) of each parameter for ketamine alone and for each CNS depressor combined with ketamine. The Shapiro Wilks W test (Table 3) shows that only the righting and exploring reflex were normally distributed.

Ataxia and Hypnosis. Ketamine alone produced only ataxia in both genders (Table 2). For the combinations, the mean time to producing ataxia was nearly one minute, and 2 min for hypnosis, earlier in females than in males (Table 2). However, no significant differences between genders were found (Table 3).

Pedal withdrawal reflex (PWR). In this parameter, the differences were between genders: the mean value for the reflex loss was faster in females (6.8 min) than in males (11.7 min) (Table 2) and these differences are significant (Table 3). There were also significant differences for the combination comparison (Table 3).

In males, the diazepam/ketamine combination did not induce loss of the pedal reflex but three out of six animals died. The animals showed a respiratory depression, observable through different types of respiratory patterns and an inability to use the thoracic muscles; respiratory movements became shallower and abdominal causing apnoea and death in three out of six cases. In females, only two out of six lost their pedal reflex but none died due to the combination with diazepam. It is important to note that ketamine combinations with aceproma-

Table 2. Statistical values (mean, *n* and s.d.) of ataxia, hypnosis, loss and recovery of the pedal reflex, recovery, righting exploratory reflex from the anaesthesia with ketamine + central nervous system depressor (CNSD) in mice (units: minutes)

CNSD	Induction		Anaesthesia		Recovery		
	ataxia	hypnosis	pedal reflex		recovery	righting	explora-tory
			loss	recovery			
Males							
Ketamine alone*	1.42 (7) 0.12						
Acepro-mazine	1.53 (6) 0.50	3.18 (5) 1.02	16.31 (5) 1.99	52.00 (5) 14.00	67.20 (5) 8.59	85.20 (5) 3.71	102.40 (5) 9.54
Diazepam	0.71 (6) 0.19	1.16 (6) 0.11			82.33 (3) 4.63	94.33 (3) 2.62	95.33 (3) 2.05
Medeto-midine	1.04 (7) 0.30	2.20 (7) 0.93	14.42 (6) 6.68	91.33 (6) 1.70	93.57 (7) 1.59	96.86 (7) 4.12	104.43 (7) 5.68
Midazolam	0.86 (7) 0.26	2.97 (7) 0.28	7.84 (7) 1.24	31.71 (7) 4.37	42.43 (7) 11.83	72.43 (7) 14.42	80.00 (7) 9.27
Xylazine	1.38 (7) 0.48	2.42 (7) 0.59	9.82 (6) 2.88	43.25 (6) 13.29	63.35 (7) 24.23	79.66 (7) 27.43	82.41 (7) 30.97
Mean values	1.10	2.17	11.74	53.73	68.22	84.54	91.93
Females							
Ketamine alone*	1.08 (7) 0.31						
Acepro-mazine	1.08 (6) 0.17	2.81 (6) 1.14			48.85 (6) 6.42	66.56 (6) 9.13	79.31 (6) 14.67
Diazepam	0.75 (6) 0.11	1.44 (6) 0.38	3.10 (2) 0.06	63.00 (2) 25.00	50.33 (6) 19.14	59.17 (6) 22.06	65.60 (5) 21.24
Medeto-midine	0.84 (7) 0.13	1.45 (7) 0.19	5.21 (7) 2.44	85.57 (7) 11.84	95.29 (7) 3.69	104.00 (7) 6.59	107.29 (7) 5.17
Midazolam	0.77 (7) 0.06	1.50 (7) 0.14			39.09 (7) 8.75	57.12 (7) 17.96	59.40 (7) 17.77
Xylazine	1.18 (7) 0.10	2.03 (7) 0.37	9.46 (7) 2.02	44.14 (7) 10.63	70.57 (7) 11.50	81.57 (7) 13.66	86.71 (7) 16.25
Mean values	0.92	1.83	6.81	64.62	61.51	74.34	80.55

*data for calculating mean values not included

zine and midazolam did not induce a loss of reflex in females. Only combinations with α_2-agonists induced pedal reflex loss in both genders in a similar way, but, in the case of medetomidine, the loss of reflex in males was later than in females.

Reflex recovery was only recorded in subjects that had lost their withdrawal reflex; there were no differences between genders, and the mean values in males and females were similar, mainly in the case of ketamine combinations with α_2-agonists (Table 3). However, the comparison between combinations always showed significant differences (Table 3). All the animals injected with the medetomidine combination were also injected 90 min later with atipamezol, despite the fact that three females had recovered their pedal reflex a few minutes prior to the administration of the antagonist. A non-parametric analysis with a Spearman rank correlation test indicated that there was no correlation ($r = 0.092$; $P > 0.05$) between the loss of PWR and its recovery, so that there was no chronological coincidence between them.

There were differences between recovery and loss of PWR, time of anaesthesia or time in which surgical procedures were allowed. Males did not reach an anaesthetic level with diazepam while females did not reach this level with acepromazine or midazolam. Statistical analysis showed that there were no significant differences between genders, but these differences were significant between combinations. Only combinations with medetomidine and xylazine reached anaesthetic level in both genders.

Recovery. The mean values for recovery (Table 2) and statistical analysis show differences between combinations but not for genders (Table 3).

Righting. For righting, the mean value for females was higher than that for males (Table 2), but, in the case of α_2-agonists, males righted themselves sooner than females. The non-parametric Kruskal-Wallis "*H*" (Table 3) shows no differences between genders while for combinations, *"H"* shows significant differences (Table 3).

Exploratory. The mean value time for females was faster than that for males (Table 2), except in the case of α_2-agonists. The Kruskal-Wallis test (Table 3) reveals differences for combinations.

The Spearman rank correlation is high for different comparisons between recovery and righting

Table 3. Test for normality (Shapiro-Wilks W test) and nonparametric statistical analysis (Kruskall-Walis H test) for comparison between sexes and associations for ataxia, hypnosis, loss and recovery of the pedal reflex, recovery, righting exploratory reflex

	Induction		Anaesthesia		Recovery		
	ataxia	hypnosis	pedal reflex		recovery	righting	explora-tory
			loss	recovery			
Shapiro-Wilks W test (H:0 for normality)							
W	0.8602*	0.8150*	0.9171*	0.8520*	0.8997*	0.9354	0.9590
P-value	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001	> 0.05	> 0.05
Nonparametric Kruskall-Walis H test							
Between genders: H	1.2347	3.0275	8.8911*	2.1847	1.1206	2.7487	2.7120
P-value	> 0.05	> 0.05	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Between associations: H	34.536*	31.4525*	13.8269*	29.3636*	37.7577*	25.6981*	23.7029*
P-value	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001	< 0.001	< 0.001

*significant differences

(0.8461; $P < 0.001$); recovery and exploratory phases (0.7850; $P < 0.001$); and righting and exploratory phases (0.8710; $P < 0.001$) showing a chronological sequence for these parameters.

Physiological parameters

Respiratory rate (RR). The respiratory rate data are not normally distributed. The representation for this physiological parameter is shown in Table 4. Data for male and female mice and their evolution time were, in appearance, similar. The most relevant observation was the increase in the RR with midazolam combination in females, in which a strong elevation of respiratory rate at 40 min was observed, after which there was a large decrease at 60 min of the control time. That elevation was seen in males at 40 min too, but, unlike the females, it was conserved at 60 min. However, the differences between genders and between times of breath control are not significant. The statistical analysis

Table 4. Respiratory rate in males and females (mean, *n* and s.d.) for the combinations of ketamine and central nervous system depressors at 10, 20, 40 and 60 min

	10 min	20 min	40 min	60 min
Males				
Acepromazine	222.8 (5) 56.6	220.8 (5) 36.5	196.8 (5) 28.6	201.5 (4) 26.9
Diazepam	144.7 (6) 60.5	97.3 (3) 19.7	114.7 (3) 72.6	140.0 (3) 25.0
Medetomidine	147.1 (7) 14.2	141.7 (7) 33.1	142.9 (7) 47.3	134.9 (7) 51.3
Midazolam	141.7 (7) 21.8	138.3 (7) 46.8	230.9 (7) 61.2	250.0 (2) 70.7
Xylazine	136.6 (7) 13.7	136.6 (7) 10.7	143.3 (6) 27.2	149.0 (4) 12.4
Mean values	158.6	146.9	165.7	175.1
Females				
Acepromazine	189.3 (6) 70.8	188.7 (6) 56.1	222.7 (6) 40.7	226.0 (2) 59.4
Diazepam	142.0 (6) 16.3	152.0 (6) 29.6	167.0 (4) 32.2	198.0 (2) 87.0
Medetomidine	128.0 (7) 20.5	119.4 (7) 15.7	108.0 (7) 19.9	99.4 (7) 16.4
Midazolam	137.7 (7) 46.4	155.6 (7) 56.3	254.7 (6) 39.4	141.3 (3) 72.6
Xylazine	119.1 (7) 22.8	100.6 (7) 17.5	99.4 (7) 29.4	114.3 (7) 37.4
Mean values	143.2	143.2	170.4	155.8

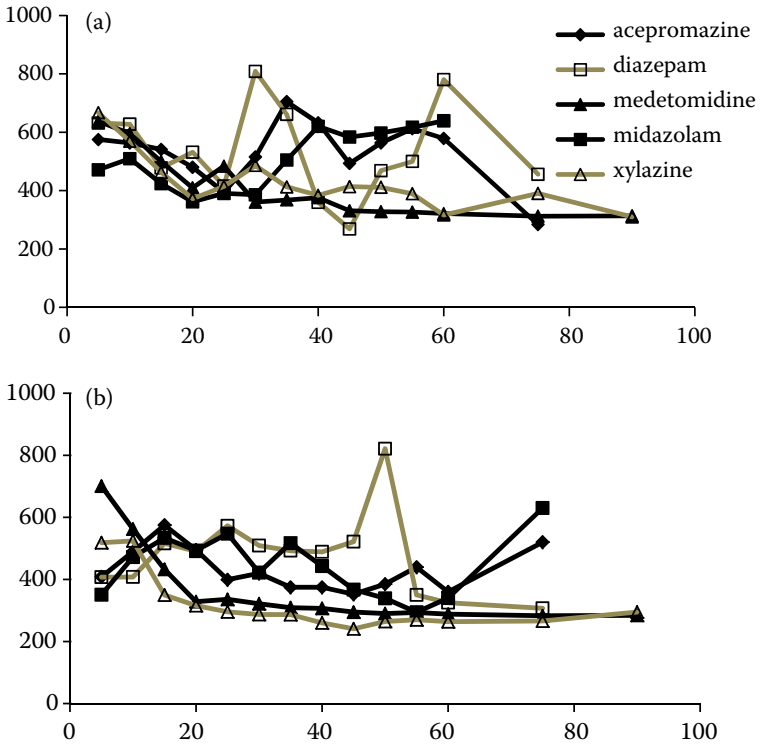

Figure 1. Heart rate in males (a) and females (b) for the combination of ketamine and central nervous system depressors at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75 and 90 min

shows an important difference in the respiratory pattern between combinations. The lowest respiratory rate occured with the diazepam combination in males and led to 50% mortality. The combinations with α_2-agonists induced a low respiratory rate in both genders but the acepromazine combination induced the highest rates.

Heart rate (HR). The graph of mean values is presented in Figure 1. The data from the heart rate are not normally distributed. It is relevant that males showed, in general, higher mean values than females at different times except at 25 and 75 min and the tendency line descends from the first to last times in males and females (Figure 1). Unlike the respiratory rate, differences for genders and for times are significant. With respect to combinations, α_2-agonist drug combinations with ketamine induced a low heart rate in both genders, whereas benzodiazepines induced higher values but very close to those of acepromazine. The statistical analysis also shows, like for the Respiratory rate, differences in the HR between combinations.

Oxygen saturation (Ox). The oxygen saturation percentage data are not normally distributed. These data are presented in Figure 2 and there are no differences either for genders or for monitored times. However, differences have been found for combinations. It is worth noting that mean oxygen saturation percentages observed for the acepromazine combination with ketamine are higher than 90% for males and females, whereas other combinations present lower percentages. The lowest percentages occur with the diazepam combination in males (producing 50% of mortality). As was established for the previous physiological parameter, α_2-agonist drug combinations induced the lowest percentage values (exception for diazepam in males).

A multiple correlation for physiological parameters in which oxygen saturation (Ox) is a dependent variable versus respiratory rate (RR) and heart rate (HR) as independent variables was studied. The fitted equation model is:

$$Ox = 68.7604 + 0.1455 \times RR - 0.0186 \times HR$$

Since the "*P*-value" in the ANOVA table is less than 0.01, there is a statistically significant relationship between the variables at the 99% confidence level ($P < 0.01$). Nevertheless, for simple models, we found that there was a correlation between Ox and RR ($r = 0.658$; $P < 0.001$) and a lower correlation between Ox and HR ($r = -0.140$; $P > 0.05$), which was not statistically significant. These results mean that the respiratory rate is the main factor for Ox, and so we can reduce the equation to:

$$Ox = 63.3761 + 0.1263 \times RR$$

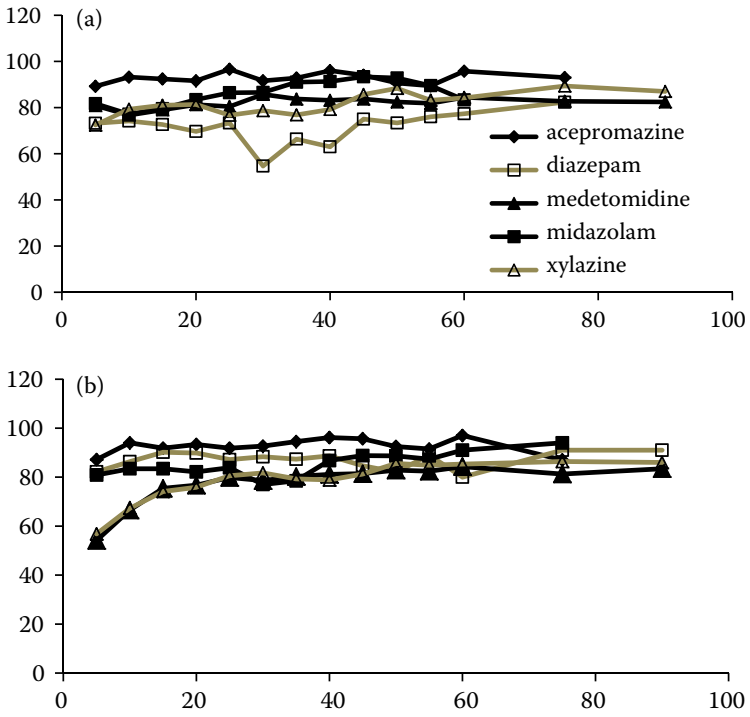

Figure 2. Oxygen saturation percentage in males (a) and females (b) for the combination of ketamine and central nervous system depressors at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75 and 90 min

DISCUSSION

The combination of some depressors with the dissociative anaesthetic ketamine remains one of the first choices for injectable rodent anaesthesia (Murray et al. 2000; Gaertner et al. 2008), due to its easy *i.p.* administration, relative safety among injectable agents, and ability to produce CNS effects such as catalepsia, analgesia, dissociation from environment and amnesia (Murray et al. 2000; Richardson and Flecknell 2005; Baker et al. 2011; Kawai et al. 2011). Plumb (2008) indicates a dose of 50–100 mg/kg of ketamine only in mice. In our study we used this dose of the product only as a reference and the data have not been included in the tables since, as was expected, hypnosis was not reached, only incoordination.

The doses of the sedatives used in this study have been in accordance with those recommended in previous investigations (Wamber et al. 1996; Hawk and Leary 1999; Flecknell 2009). Preliminary studies by different authors have reported that medetomidine induces a long period of sedation with a severe decrease in the respiratory rate and in arterial oxygen saturation; in order to maintain the beneficial effects we have reversed to medetomidine as has been recommended (Cruz et al. 1998; Henke et al. 2004; Hahn et al. 2005). Given that Baker et al. (2011) report that the administration of atipamezole over a short time may lead to a "re-sedation effect", we established a time of 90 min after the medetomidine injection, for the administration of the antagonist. Due to hypothermia possibly being one of the most frequent causes of mortality (Flecknell 2009), once animals went into hypnosis, they were placed on a homeothermic blanket.

It is important to note that, in the case of the diazepam combination in males, death occurred in half of the animals (three out of six), and the other half did not reach an anaesthetic level because they did not lose their PWR. We did not find any specific references on this difference between genders, but Gaertner et al. (2008), indicated a higher mortality for the diazepam/ketamine combination within 15 min of induction with a combination at 7.5–10 mg/kg diazepam/60–80 mg/kg ketamine, and commented that higher mortality was observed when compared with other multimodal anaesthesia methods. In spite of this, the diazepam/ketamine combination is frequent in the anaesthesia of different animal species (Flecknell 2009), although it has been reported that it can cause respiratory depression and death. However, diazepam was used along with the other depressors with the aim of comparing them, since the bibliographic references recommend the diazepam/ketamine combination, among others (Murray et al. 2000; Plumb 2008; Flecknell 2009).

This protocol was not suspended because the observer-researcher received the animals sequen-

tially before the deaths of the three animals began to occur, the group of males to which the diazepam/ketamine combination was administered being precisely the last one to be evaluated.

We agree with (Cruz et al. 1998; Arras et al. 2001; Orr et al. 2005) and recommend the α_2-agonist combination due to the known analgesic action of this group of drugs, in spite of the combination of xylazine/ketamine as an anaesthetic possibly being combined with an insufficient analgesia in mice (Smith 1993; Flecknell 2009). As a result, only the α_2-agonist/ketamine combination seems to be adequate, at these doses, for use as an anaesthetic because it prevents pain perception in the animals, the data being the same as those previously reported by other authors (Cruz et al. 1998; Arras et al. 2001). Our results from the xylazine/ketamine combination (10/100 mg/kg) were very similar to those of other authors as regards the data obtained on the duration of time of reflex absence and the time of recovery of the reflex, although at a somewhat lower concentration (8/80 mg/kg) than that used by us (Kawai et al. 2011). In the case of these same authors using an even lower concentration (6/60 mg/kg), the duration of the absence of reflex and the moment of its recovery were shorter, thus confirming that the effect of combinations of xylazine and ketamine depends on the quantities of these drugs (Kawai et al. 2011).

In agreement with Cruz et al. (1998), we also found that the females recovered later with the medetomidine/ketamine combination than males, but these differences were not significant. A comparison between combinations shows that medetomidine induces a longer period of anaesthesia in both genders. Likewise, a similar mean duration of anaesthesia with midazolam and medetomidine combined with ketamine in rabbits at different doses has been reported by Grint and Murison (2008).

Since the time to normal locomotion is a biologically important parameter, because the animal starts consuming food and water again normally, as well as managing to establish homeostasis more rapidly (Baker et al. 2011), and because medetomidine tends to produce more prolonged periods of anaesthesia, we reversed the medetomidine/ketamine combination at 90 min with atipamezol, as mentioned above.

The scant respiratory depression with the acepromazine combination is in agreement with the data reported by Flecknell (2009). This depression produces a small reduction in the blood oxygen saturation level. On the other hand, α_2-agonists have a more depressed respiratory rate and blood oxygen saturation level as a consequence of the well-known sympathetic neuro-vegetative depression at the presynaptic level, and induce a low level of blood oxygen saturation. Thus, perhaps oxygen could be recommended as a supplement to the room air for the duration of the procedure. In our study, no oxygen supplement was used as we tried to seek a combination which would produce the best anaesthesia conditions in mice, with the least amount of resources available, since many research centres may not possess the necessary equipment. Moreover, small animal clinics, if they are not specialized in exotic animals, usually do not have specific equipment for rodents, or, more specifically, for mice.

With respect to heart rate reduction, α_2-agonists also induce this effect due to their sympathetic neurovegetative depression, although higher HR values have been reported by Sirosis (2005). The other combinations produce a small reduction in HR (Arras et al. 2001; Orr et al. 2005). In males, the HR reduction is lower than in females. There is a significant correlation, both for respiratory rate and oxygen saturation, between xylazine versus medetomidine implying a similar action for both α_2-agonists. With regard to benzodiazepines, it is interesting to note that diazepam reduces RR and Ox in males more than other drugs and produces a mortality of 50% in them. For this reason, we were not able to study the diazepam versus midazolam correlation for RR or Ox.

In conclusion, our results have demonstrated that combinations of ketamine with α_2-agonists induce best results in both genders at the doses that we have studied, but there is an important sympathetic neurovegetative depression and it must be reduced by including other drugs in anaesthesia protocols, in addition to oxygen supplementation.

On the other hand, it has been verified that the combination of ketamine with acepromazine, diazepam or midazolam does not produce a good anaesthesia in mice (males and females) so these combinations are not recommendable, at least at these doses, or combined with other drugs.

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