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Anesthetic effects of a mixture of xylazine, ketamine, and buprenorphine in laboratory rats subjected to short surgical procedures

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ABSTRACT

Background: Rodents are commonly used as models in experimental procedures, and researchers often need to perform rapid manipulations involving sedation and analgesia.

Aim: The aim of this study was to evaluate the validity of the combination of xylazine and ketamine in association with buprenorphine in experimental rats undergoing short-term surgical procedures.

Methods: Twenty-six male rats were enrolled in experiments. Thirty minutes before the start of the procedure, buprenorphine (0.05 mg/Kg) was administered subcutaneously. The sedative protocol included intraperitoneal (IP) administration of 70 mg/Kg ketamine and 10 mg/Kg xylazine. Additionally, at the end of the procedure, all rats received 0.1 mg/Kg of atipamezole IP. Immediately before sedation and at 5, 10, 15, and 20 minutes after atipamezole administration, the main cardiorespiratory parameters were recorded. In addition, induction time, depth of anesthesia, duration of the procedure, recovery time, and pain score were recorded.

Results: The mean induction time was 2.29 ± 0.95 minutes. At the time of surgery, all subjects showed a deep anesthetic plane (score ≥ 3), and no response to skin incision was observed (score = 0). The time to recovery from the righting reflex after atipamezole administration was 3.66 ± 1.09 minutes. No rats showed signs of pain based on the rat Grimace scale.

Conclusion: Our results suggested that the association of opioids with the xylazine/ketamine protocol ensures rapid induction and good analgesia during short procedures with mild/moderate painful stimulation. Furthermore, the administration of atipamezole facilitates rapid recovery and resumption of motor activity.

Keywords: Rats, Xylazine/ketamine, Buprenorphine, Short-term procedures, Atipamezole.

Introduction

Rats are commonly used in scientific research, and experimental procedures often involve surgery in which anesthesia and analgesia are required and recommended (Cicero *et al*., 2018). In recent years, various anesthetic protocols have been described in experimental rodents, and the choice of a specific protocol is influenced by several aspects, such as age, health status, type and duration of the procedure, and the desired recovery time (Dittman *et al*., 2004; Stokes *et al*., 2009; Salice *et al*., 2013). In addition, it is necessary to consider some peculiar aspects of rodents, including their small size, accelerated metabolism, and easy development of hypothermia, which make anesthesiologic management complex and worthy of attention (Gargiulo *et al*., 2012; Albrecht *et al*., 2014A; Alemán-Laporte *et al*., 2020). Short-term manipulations and procedures on large numbers of rats are performed daily, and the application of anesthetic protocols that include minimal stress, rapid sedation, and awakening with immediate recovery of major organ functions should be the gold standard Alemán-Laporte *et al*., 2020. Furthermore, the addition of analgesic drugs reduces pain during procedures, representing a key point of the "3R" principle (Roughan *et al*., 2000; Roughan *et al*., 2001; Roughan *et al*., 2003). The main methods of induction and maintenance of anesthesia in rats include the use of halogenated gases and/or injectable anesthetics. The administration of halogens involves the use of an induction chamber or face mask. Typically, isoflurane and sevoflurane are used, although isoflurane, despite its pungent smell, is cheaper and ensures faster induction (Buitrago *et al*., 2008; Cicero *et al*., 2018). The advantages of this approach include: 1) greater operator control over the depth of anesthesia; 2) rapid absorption and elimination; 3) direct action on GABA (gamma-aminobutyric acid) and glycine receptors, resulting in immobility, hypnosis, and muscle relaxation (Rislin *et al*., 2012; Oh and Narver, 2024). On the other hand, there are several disadvantages

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to consider, such as respiratory and blood pressure depression, the requirement of heavy and expensive equipment (e.g., properly calibrated flowmeters and vaporizers), potential environmental pollution, and the high risk of anesthetic gas inhalation by the operator (Oh and Narver, 2024). Furthermore, considering the small size of the rats, the equipment can be uncomfortable during surgical procedures, especially when performing procedures involving the head and neck (e.g., tumor or neuroscience studies) (Buitrago *et al*., 2008). Injectable anesthetics are particularly useful when short surgical procedures need to be performed on a large group of animals (Oh *et al*., 2024). For these reasons, over the years, numerous anesthesiologic protocols have been studied for use in experimental rodents. Among these, the most frequently used drugs are: α_2-agonists, benzodiazepines, ketamine, and opioids (Buitrago *et al*., 2008; Kawai *et al*., 2011; Tsukamoto *et al*., 2018; Oh and Narver, 2024). Ketamine is a noncompetitive NMDA receptor antagonist that is widely used to induce anesthesia and analgesia. It ensures the maintenance of good vascular tone and heart rate (Annetta *et al*., 2005). However, ketamine can have adverse effects, including agitation upon awakening and dysphoric events (Zhou *et al*., 2021). For these reasons, it is commonly associated with an α_2-agonist such as xylazine (XK) (Albrecht *et al*., 2014A; Cicero *et al*., 2018; Hohlbaum *et al*., 2018). Among the various combinations proposed in the literature, it has been shown that the addition of acepromazine to a protocol with XK causes hypotension and prolongs the duration of anesthesia (up to approximately 70 minutes) (Alemán-Laporte *et al*., 2020). Alternatively, the use of midazolam, medetomidine, and fentanyl seems to ensure good analgesia and an anesthesiologic plan, but causes greater hypertension and bradycardia compared with XK, leading to a greater risk of intraoperative bleeding (Albrecht *et al*., 2014A; Fleischmann *et al*., 2016). Albrecht *et al.* (2014AB) reported that although the XK protocol ensures greater hemodynamic stability, long recovery times require prolonged monitoring. Contextually, the authors suggested that the addition of an antagonist could accelerate recovery times (Albrecht *et al*., 2014A). Regarding the analgesic effect, during surgical procedures, several studies suggested the addition of an opioid. Among the opioids described, buprenorphine is the most commonly used in experimental rats, considering its good analgesic effect, long duration of action, and few respiratory side effects (Goldkuhl *et al*., 2010; Guarnieri *et al*., 2012; Houston *et al*., 2021).

Based on the information gathered from the extensive literature presented in previous years, our considerations are as follows: 1) the XK protocol can be used for short-term procedures, but a quicker recovery and better quality of awakening is obtained if the α_2-agonist is antagonized; 2) the addition of an opioid such as buprenorphine during painful procedures ensures good intra- and postoperative analgesia. Considering this, the aim of this study was to set up and evaluate a valid anesthesiologic protocol to be applied in experimental rats subjected to short-term surgical procedures, which guarantees rapid sedation, quick recovery, and good analgesia throughout the perioperative period. To this end, the subjects included in the study were monitored during the pre-, intra-, and post-operative periods, considering physiological parameters, anesthesia, and surgery times, and a post-operative pain assessment score (Rat Grimace scale) (Sotocina *et al*., 2011; Leung *et al*., 2016).

Materials and Methods

Animal housing

Twenty-six male Wistar rats weighing 300–400 g and aged 4–5 months were enrolled to perform a translational experimental project. The subjects were obtained from Charles River Laboratories Italia S.r.l. (Calco, Italy) and acclimated to the University of Camerino animal facility for 7 days before carrying out the experimental procedures. Rats were divided into groups of two to ensure physiological socialization of the species and placed in Plexiglas cages at room temperature (20°C–22°C, 45%–55% humidity) and a 12-hour light-dark cycle. All subjects were fed specific food pellets (4RF Mucedola, Settimo Milanese, Italy) and were provided water ad libitum. Subjects with respiratory symptoms, loss of appetite, weight loss, and skin or hair changes were excluded from the study a priori.

Anesthetic protocol

All rats received the same anesthetic protocol, and they were adequately weighed to ensure proper drug administration. Thirty minutes before the start of the procedures, buprenorphine (0.05 mg/Kg; Buprefelican 0.3 mg/ml, Dechra S.r.l, Italy) was administered subcutaneously (Oh and Narver, 2024). Considering the small size of the subjects and to ensure greater precision of the injected volume, 0.1 ml of buprenorphine was diluted with 0.9 ml of NaCl 0.9% (Sodium Chloride 0.9%, BBraun, Italy), resulting in a drug concentration of 0.03 mg/ml in a total volume of 1 ml (1:10 ratio). The sedative protocol included intraperitoneal (IP) administration of 70 mg/Kg of ketamine (Lobotor 100 mg/ml, Acme S.r.l., Italy) and 10 mg/Kg of xylazine (Nerfasin 20 mg/ml, P.H. Farmaceutici S.r.l., Italy) (Oh and Narver, 2024). These two drugs were mixed in a sterile 1 ml syringe with a 25-gauge hypodermic needle and injected into the right caudal quadrant of the ventral abdomen to avoid iatrogenic damage to the cecum. At the end of the surgery (after the last suture was applied), all rats received intraperitoneal administration of 0.1 mg/Kg atipamezole (Antisedan 5 mg/ml, Zoetis S.r.l., Roma, Italy) and were placed on a heating pad (Oh and Narver, 2024). To obtain a volume of atipamezole suitable for administration, 0.1 ml of the drug was diluted with 9.9 ml of NaCl 0.9%

(Sodium Chloride 0.9%, BBraun, Italy), resulting in a final concentration of 0.05 mg/ml (1:100 ratio). Then, rats were handled gently with a towel to minimize the containment stress and were placed in a single cage until the loss of the right reflex. Furthermore, during the sedation and recovery phases, supplemental pure oxygen was administered via a face mask.

Pre- and intraoperative assessments

Immediately before IP injection (T_{pre}), body temperature (T_{body}; C°), heart rate (HR; beats/minutes), and respiratory rate (RR; breaths/minutes) were recorded. T_{body} was assessed using a rectal temperature probe. HR was calculated using a stethoscope by quadrupling the number of contractions detected in 15 seconds. Similarly, the RR was calculated by quadrupling the number of thoracic excursions observed in 15 seconds. Following the administration of sedative drugs, a 5-point score (0–4) was used to assess the depth of anesthesia based on reflex responses. Specifically, the thoracic and pelvic limb withdrawal, eyelid, and tail jerk reflexes were studied (0 = presence of all 4 reflexes; 1 = loss 1 of 4 reflexes; 2 = loss 2 of 4 reflexes; 3 = loss 3 of 4 reflexes; 4 = loss of all reflexes) (Tsukamoto *et al*., 2018).

The withdrawal reflex was elicited by applying pressure with forceps to the forelimb and hindlimb, the eyelid reflex by touching the medial canthus of the eye with the finger, and the tail reflex by pinching the tail with the same forceps. Nociceptive stimuli were applied by the same operator using atraumatic forceps to reduce pressure variability. A score ≥ 3 indicated a sufficient depth of anesthetic to begin the surgical procedure, whereas a score < 3 indicated an excessively low level of anesthetic. Monitoring was performed starting 60 seconds after IP puncture and then every minute until a score of 3 or higher was achieved. Subsequently, the duration of surgery (from the surgical incision to the placement of the last suture) was recorded (Surgery Time). The reaction to surgical stimulation was assessed according to the reflex in response to the skin incision (0 = no response; 1 = mild response; 2 = massive response) (Tsukamoto *et al*., 2018).

Pure oxygen was administered via a face mask during the entire procedure, and partial oxygen saturation (SpO_2; %) was continuously monitored by placing a pulse oximetry probe (VE-H100B Edan, Alcyon Italy S.p.a, Cherasco, Italy) on the toes of the hind limb.

Surgery procedure

The primary objective of the present study was to evaluate the biocompatibility of the human acellular dermal matrix in an animal model. Hence, rats were positioned in the sternal recumbency region, and the hair of the infrascapular region was clipped. The skin of the surgical site was disinfected with 10% povidone-iodine 10% and ethyl alcohol 95%. Aseptically, an infrascapular skin incision (about 2 cm) was made, and the biomaterial was implanted in the hypodermal layer. Subsequently, the skin was sutured using USP 4/0 polydioxanone thread (Ethicon, Somerville, NJ).

Postoperative assessments

At the end of the surgery, atipamezole was administered as previously described, and the main physiological parameters (Spo_2, HR, RR, and T_{body}) were concurrently recorded. The RR and T_{body} were also monitored every 5 minutes after atipamezole administration for 20 minutes (T5, T10, T15, and T20, respectively).

The quality and time of recovery were assessed using a five-point Likert-type scale. The score includes the assessment of the ability to maintain the quadrupedal station, the presence of ataxia (identified as the tendency to fall to the right or left during walking), and the presence/absence of the righting reflex, palpebral reflex, and limb withdrawal reflex (Hohlbaum *et al.,* 2018). The latter was assessed by applying pressure with a forceps to the extremity of the right hind limb. Specifically, a score ≤ 1 suggested a good quality of recovery characterized by the return of locomotory activity in the absence of ataxia and falls (Table 1) (Lee *et al*., 1998).

The duration of anesthesia was defined as the time between loss of righting reflex and return of locomotor activity (recovery score < 2), while recovery time was defined as the period from subcutaneous injection of atipamezole (T0) to return of locomotor activity. In addition, the total duration of the procedure (time between IP injection and return of locomotor activity) was recorded. All study times are shown in Figure 1.

Postoperative pain was assessed using the Rat Grimace Scale 30 minutes after atipamezole injection (TGrim0) and 3 (TGrim1) and 6 hours (TGrim2) after TGrim0 (Sotocina *et al*., 2011). In cases with a score indicative of pain (score > 1), rescue analgesia (50 µg/Kg of buprenorphine SC) was administered.

Table 1. Five-point Likert-type scale used to evaluate the quality of recovery after surgery.

Score	Description
0	Ability to walk in the absence of ataxia and falls. All reflexes are present.
1	Presence of righting reflex. Ability to maintain quadrupedal posture. Ataxia and lateral falls.
2	Head raising. Presence of righting reflex but inability to maintain quadrupedal stance.
3	No head movement. Absence of righting reflex. Eyelid reflex and pinch withdrawal reflex
4	No movement. Absence of eyelid reflex and pinch withdrawal reflex.

Statistical analysis

Statistical analysis was performed using MedCalc software 9.0 (MedCalc version 9.2.10). All data resulted normally distributed based on the Shapiro–Wilk test. Physiological parameters were evaluated using the one-way ANOVA test to perform a comparison between times. All results are reported as mean ± standard deviation. Differences with a p value < 0.05 were considered statistically significant.

Power calculations and the primary study aim

The aim of the primary study was to evaluate the inflammatory response, immunological properties, and integrative capacity of decellularized membranes obtained through tissue engineering from human donor tissue.

The sample size calculation was performed using Prudente *et al.* 2016. To obtain the correct sample unit, G*Power Version 3.0.10 software was used at the University of Düsseldorf, Germany (Faul *et al*., 2009). The analysis was performed using two different parameters to obtain greater reliability in the sample selection process. From the evaluation of interleukin 1, the sample power calculation indicated a "power" of 0.95, an "effect size" of 1.85, and an alpha error of 0.05. The extrapolated results from the analysis suggest that a minimum number of 8 rats per study group is sufficient to obtain statistical significance. From the evaluation of tumor necrosis factor alpha, the calculation of the sampling power indicated a "power" of 0.95, an "effect size" of 1.36, and an alpha error of 0.05. The extrapolated results from the analysis suggest that a minimum of 13 rats per study group are sufficient to obtain sampling significance. The two parameters were compared 4 and 30 days after tissue implantation, as described in the literature. The one-tailed t-test was used. An a priori analysis was used to evaluate the differences between the two independent means. It was, therefore, decided to use 2 groups of 13 animals (a total of 26) because the number of the two groups would have enough power to guarantee a sample significance for the different inflammatory factors to be analyzed.

Ethical approval

This study was approved by the Italian Ministry of Health (protocol number 424/2021-PR) in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Results

The mean weight of the animals on the day of surgery was 349.69 ± 93.46 g. Two rats were excluded due to incorrect intraperitoneal administration of the anesthetic drugs. The remaining 24 subjects survived the surgical procedures and completed the study. This study conforms to the Consolidated Standards of Reporting Trials (CONSORT) Statement 2010 for reporting randomized trials (Moher *et al*., 2010) (Fig. 2).

Reflexes and times recorded

The mean total procedure duration was 17.87 ± 1.91 minutes, while the surgery time was 3.2 ± 1.16 minutes. The mean time from intraperitoneal injection

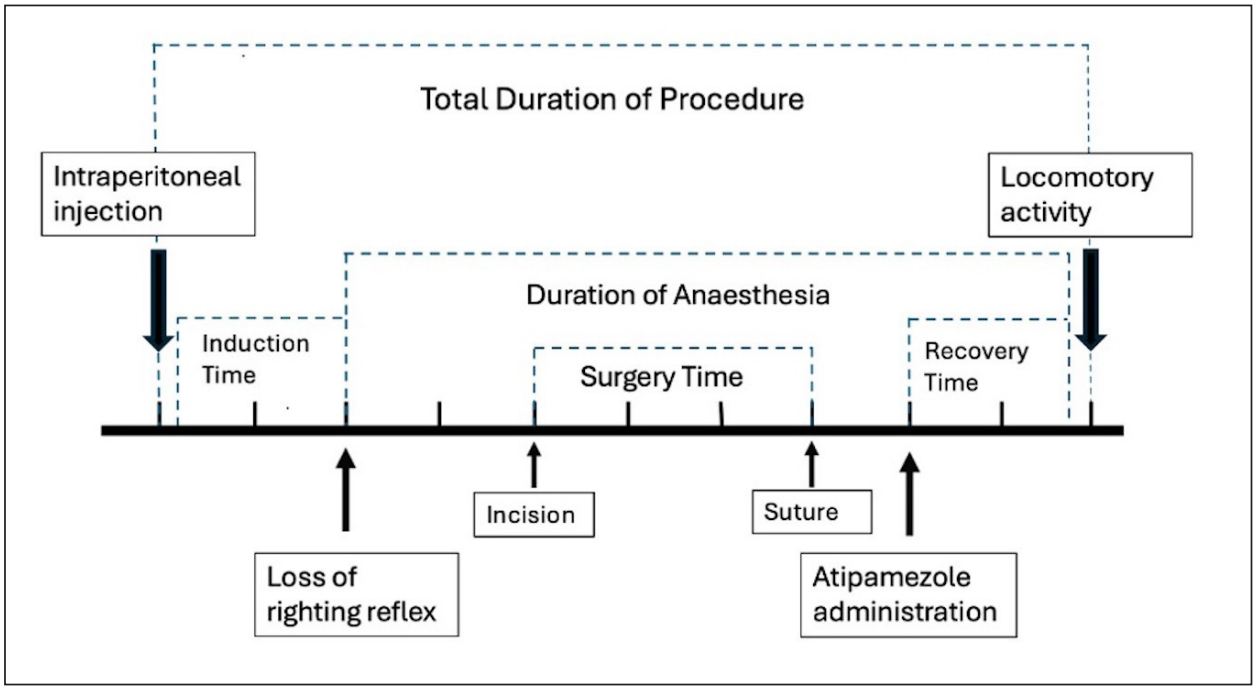

Fig. 1. Schematic representation of the times recorded during the experimental study.

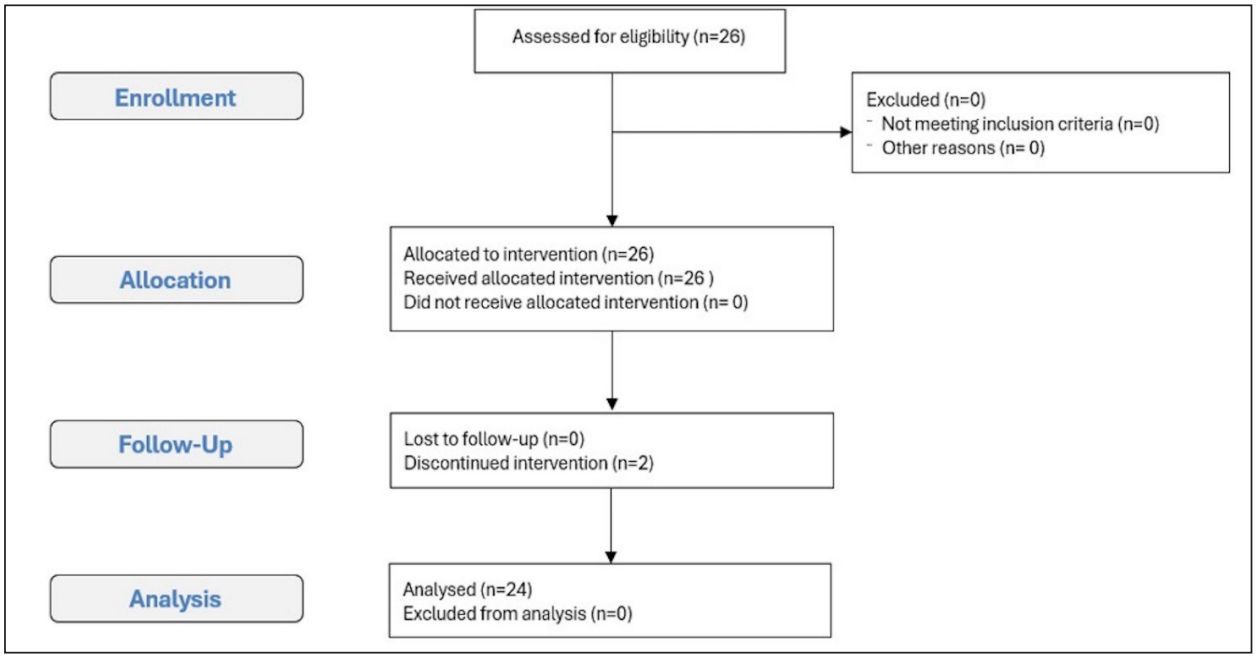

Fig. 2. Consolidated standards of reporting trials flow diagram of the rats included in the experimental study.

to loss of righting reflex (Induction Time) was 2.29 ± 0.95 minutes. At the time of surgery, all subjects showed a deep anesthetic plane (score ≥ 3), and no response to skin incision was observed (score = 0). The mean time from loss of righting reflex to return of locomotor activity (Duration of Anesthesia) was 14.5 ± 1.94 minutes. The period from atipamezole injection to return of locomotor activity (Recovery Time) was 3.66 ± 1.09 minutes. At T0, all rats showed an absence of spontaneous activity and loss of righting reflex (Recovery Score ≥ 2), whereas at T5, they returned to spontaneous activity (Recovery Score < 2) (Table 2).

Physiological parameters

HR decreased significantly at T0 ($p < 0.05$) compared with baseline (Tpre). After 5 minutes from atipamezole administration (T5), the heart rate increased significantly compared with T0, reaching values comparable to Tpre and remaining stable at subsequent times T10, T15, and T20. Similarly, RR was lower at T0 than at Tpre; however, no statistical differences were observed between the various study times ($p > 0.05$). Body temperature decreased significantly at all study times compared with Tpre ($p < 0.05$). Subsequently, at T15 and T20, it increased significantly compared with T5, but without reaching baseline values. Regarding SpO_2 values, the mean was 99% ± 0.32 % and 98% ± 0.45 % at Tsurg and T0, respectively. All of the above parameters are reported as mean ± standard deviation in Table 3. No subject received rescue analgesia, and the Rat Grimace Scale showed score = 0 at TGrim0, TGrim1, and TGrim2.

Discussion

In this study, the application of the anesthesiologic protocol consisting of xylazine/ketamine/buprenorphine in short-term surgical procedures in experimental rats was described. From the data obtained, we can state that the above-mentioned protocol guarantees the following: 1) rapid sedation after intraperitoneal inoculation; 2) suitable anesthetic depth during short-term procedures that involve mild/moderate nociceptive stimulation; 3) rapid awakening, thanks to the antagonization of the α_2-agonist by administration of atipamezole.

Regarding the induction phase, all subjects achieved a sedation score ≥ to 3 within 3 minutes of drug administration. The X/K association has been described in several studies; however, there are conflicting opinions regarding its efficacy (Stokes *et al*., 2009). Albrecht *et al*. (2024AB) highlighted an induction time of about 4 minutes; however, tolerance to surgery was evidenced only after 10 minutes from drug administration (Albrecht *et al*., 2014A). It is the opinion of the authors that the addition of buprenorphine in our protocol has induced a synergism of potentiation with the other drugs favoring a deeper anesthetic level in faster times and good analgesia during nociceptive stimulations (Curtin *et al*., 2009). The absence of an analgesic in an anesthetic protocol could, therefore, lead to a greater response to the induced painful stimulations (Hedenqvist *et al*., 2000; Oh and Narver 2024). It should be noted, however, that the nature of buprenorphine (partial μ agonist) makes it an excellent analgesic for the management of mild/moderate pain

Table 2. Mean ± SD of different times (minutes) registered during the experimental procedure.

Total duration	Induction time	Surgery time	Duration of anesthesia	Recovery time
17.87 ± 1.91	2.29 ± 0.95	3.2 ± 1.16	14.5 ± 1.94	3.66 ± 1.09

Table 3. Mean ± SD of physiological parameters. [a]$p < 0.05$ differences compared with Tpre. [b]$p < 0.05$ differences compared with T0. [c]$p < 0.05$ differences compared with T5.

	HR (beats/minutes)	RR (breaths/minutes)	Tbody (C°)
Tpre	343.38 ± 59.57	93.44 ± 27.8	37.33 ± 0.74
T0	213.38 ± 53.43[a]	84.03 ± 25.01	36.50 ± 0.64[a]
T5	288.32 ± 38.63[ab]	94 ± 21.94	36.13 ± 0.48[ab]
T10	320.23 ± 42.94[abc]	95.23 ± 20.34	36.20 ± 0.34[ab]
T15	330.07 ± 37.68[abc]	96.69 ± 20.34	36.48 ± 0.24[ac]
T20	331.76 ± 45.86[abc]	94.60 ± 16.91	36.60 ± 0.39[ac]

but may not be sufficient for the management of severe nociceptive stimuli, in which the administration of pure μ agonists is more recommended (e.g., fentanyl and methadone) (Arenillas et Gomez de Segura, 2018; Alemán-Laporte *et al*., 2020).

Another aspect that we highlight is that the respiratory rate did not show statistically significant differences in the study phases. Hedenquivist *et al* (2000) reported two deaths following the administration of buprenorphine in experimental rats. However, they did not show significant differences in the respiratory rate, but they were associated with mortality in severe hypoxic states, recommending always administering supplemental oxygen after opioid administration (Hedenquivist *et al*., 2000). In this study, we did not highlight similar problems; however, the subjects were oxygenated throughout the procedure, and SpO_2 was monitored. The authors, therefore, recommend that supplemental oxygen be always provided to patients receiving anesthetic drugs, especially if the protocol includes opioids.

The HR decreased significantly at T0 and then gradually increased until it returned to baseline. The reasons for this decrease are probably: 1) the bradycardic effect of the α_2-agonist (the baroreceptor reflex triggered by the marked elevation of blood pressure) and 2) the achievement of an adequately deep anesthetic plane (Sano *et al*., 2013). Unfortunately, due to the very short surgical times and the need to respect the times required by the primary experimental study, we could not adequately monitor arterial blood pressure (ABP). This is a limitation of our study. Albrecht *et al*. (2024AB) did not find alterations in HR using the XK protocol; however, they used lower (half) xylazine doses than us, which could have influenced the result (Albrecht *et al*., 2014B). On the other hand, in confirmation of this, other authors, using our xylazine dosage (10 mg/Kg) in experimental rats, showed significant bradycardia (Picollo *et al*., 2012; Sano *et al*., 2013; Navarro *et al*., 2021). Another consideration is that the addition of opioids may have further favored the decline in heart rate (Mahinda *et al*., 2004).

Body temperature significantly decreased after the administration of the anesthetic protocol. Maintaining a good body temperature is extremely important in these animals, and unfortunately, many anesthetics cause hypothermia by inhibiting central and peripheral thermoregulatory mechanisms; consequently, loss of thermal homeostasis could directly contribute to an increase in mortality in laboratory rodents (Wixson *et al*., 1987). The use of the X/K protocol may directly influence the decrease in body temperature for the aforementioned reasons (Redfors *et al*., 2014). The authors did not highlight temperatures below 36°C in the present study; however, it should be considered that the total duration of the procedure was very short. Based on these evaluations, it is our opinion that during short procedures, the X/K protocol does not have a high impact on body temperature. However, longer procedures could involve greater risks, and it is, therefore, recommended to always use thermal supports during experimental procedures.

All subjects regained walking ability approximately 4 minutes after the administration of atipamezole. Rapid recovery from general anesthesia ensures less stress and rapid resumption of feeding and, in general, of major organic functions. In addition, it guarantees faster work in less time. The use of atipamezole in rats is still debated because it has been shown that, due to its cytochrome P450 metabolite, its pharmacokinetics are nonlinear, unlike that in humans and other species. On the other hand, clinical and experimental studies have

defined the effective dose range in rats of 0.1–1 mg/Kg administered SC or IP (Navarro *et al*., 2021; Bennett et Lewis, 2022; Oh and Narver, 2024). Furthermore, several studies have shown that during the recovery phase from general anesthesia, atipamezole antagonizes the action of xylazine better than yohimbine, inducing a rapid return of the righting reflex and a normal heart rate (Janssen *et al*., 2017). These aspects are extremely important in experimental rats because they reduce the risk of peri-anesthetic mortality (Brodbelt *et al*., 2008). Furthermore, another advantage to consider is that the administration of atipamezole does not alter the analgesia induced by opioids (e.g., buprenorphine) (Izer *et al*., 2014). For these reasons, unless there are reasons that induce the operator to maintain a longer recovery phase, the authors recommend antagonizing α_2-agonists at the end of the anesthetic procedures in experimental rodents using previously described doses of atipamezole.

As previously mentioned, this study has some limitations. ABP was not measured during the perioperative period. It would have been interesting to evaluate the arterial pressure changes induced by the anesthetic protocol used and to relate them to the HRs detected at the different study times. Another aspect to consider is that HR and RR were calculated by an operator through cardiac auscultation and observation of thoracic excursions, respectively. This could lead to operator-dependent alterations with consequent underestimation of the recorded values (considering for example the high physiological HR of rats). The use of instrumental methods (such as electrocardiogram) would have made the recorded data more reliable. Another limitation to consider is the absence of a control group to include in our study design. Unfortunately, having to comply with the protocols defined in the ministerial authorization, we could not use other drugs; however, the study would have been more complete if our protocol had been compared with a control group or with different protocols.

Conclusion

The X/K anesthesia protocol with the addition of buprenorphine appears to be optimal for the execution of short and rapid procedures in experimental rats. Moreover, the addition of opioids guarantees good analgesia during mild/moderate painful stimulations. Furthermore, the use of atipamezole as an antagonist of the α_2-agonist at the end of the procedure guarantees rapid awakening and rapid resumption of motor activity.

Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

Luca Pennasilico: study execution, data analysis, and writing of the first draft of the manuscript. Federica Serino and Margherita Galosi: study execution, data collection. Angela Palumbo Piccionello, Alessio Angorini, and Fabrizio Dini: analysis and interpretation of the data, reviewing, and editing. Caterina Di Bella: study design, data interpretation, review, and editing. All authors have read and approved the final version of the manuscript.

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Data availability statement

The corresponding author (email: caterina.dibella@unicam.it) is available to be contacted to request additional material and raw data relating to the presented study.

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