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Ketamine–Xylazine–Acepromazine Anesthesia and Postoperative Recovery in Rats

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We evaluated the effect of ketamine–xylazine–acepromazine anesthesia (31.25, 6.25, and 1.25 mg/kg subcutaneously, respectively) on postsurgical recovery in male Sprague–Dawley (Crl:SD) rats undergoing laparotomy with and without the postoperative analgesic ketorolac. Recovery was determined by changes in body weight (BW) and water intake. The time of ketorolac administration (5 mg/kg intramuscularly), 60 min after anesthetic injection, was based on return of the pedal withdrawal reflex in Long–Evans (HsdBlu:LE) rats undergoing stereotaxic surgery in a separate experiment. Results were compared with those of housing and anesthesia controls as well as of laparotomized rats receiving a single sugared treat for nonpharmacologic management of postoperative pain. Surgery took place on day 0; the first 24 h postsurgery was considered the 'acute phase,' and days 1 through 4 comprised the 'recovery phase.' Results suggest that 1) the anesthetic mixture is fast- and long-acting and provides sufficient immobility, loss of consciousness, and analgesia; 2) during the acute phase, rats subjected to laparotomy did not lose more BW than rats exposed to anesthesia alone; 3) water intake during both phases did not significantly differ between treatment groups; 4) postsurgical ketorolac administration did not minimize BW loss during the acute phase nor cause any adverse effects under this anesthetic regimen; and 5) provision of single sugared treats had salutary effects on BW recovery. This finding suggests that postsurgical BW loss after use of this anesthetic mixture is due to distress unrelated to pain; this nonpain distress may have masked potential beneficial effects of ketorolac.

Abbreviations: BW, body weight; HPA, hypothalamic–pituitary–adrenal; PWR, pedal withdrawal reflex

Surgical manipulations are an important part of many studies designed to evaluate neurobiology, pharmacology, and behavior. In our laboratory, rats subjected to adrenalectomy,[63] jugular vein catheterization,[56] and stereotaxic surgery[60] are used routinely in studies designed to evaluate central nervous system regulation of the hypothalamic–pituitary–adrenal (HPA) axis, with reference to the etiology or pathophysiology of anxiety disorders.[33,57] However, the tissue damage that accompanies any form of surgery leads to pain and distress,[26] and the stimulation of specific pain pathways can activate the HPA axis even in anesthetized animals and potentiate HPA axis responses to other stressors.[4,5] Clearly, pain is a strong aversive stimulus that can act as either a chronic stressor or a factor to sensitize key neurocircuits and thereby bias experimental outcomes.[2,32,36,45,53,62]

Analgesics are the primary choice for control of pain. Studies demonstrating their beneficial effects on postoperative recovery generally are done in rats subjected to laparotomy under inhalation anesthetics, which lack pre-emptive analgesic properties.[50,55] Furthermore, the effects of inhalant anesthetics last only for a short time, and when 'coming out' of the anesthetics, rats exhibit severe pain-related behaviors,[49,50] which can lead to reduced food and water intake and prolonged postsurgical recovery.[10,13,15,35] Many of these effects are ameliorated with the use of pre- or postoperative (or both) administration of analgesics.[9,10,13,15,35,66] However, some analgesics have adverse effects on postoperative recovery[22,28,29,52] and potentially can interfere with experimental outcomes.

An injectable anesthetic mixture that our laboratory uses routinely for surgical manipulations of rats in neuroendocrinologic studies[33,56-58] is composed of ketamine, xylazine, and acepromazine and provides muscle relaxation, loss of consciousness, and analgesia.[34,57] Accordingly, this combination can be considered a 'balanced anesthetic'—a term used to indicate an anesthetic regimen that makes use of a combination of drugs, each in an amount sufficient to produce its predominant or desired effect to the optimum degree while reducing the potential adverse effects associated with the individual agents.[27,31] The analgesic properties of the individual components of the ketamine–xylazine–acepromazine mixture have been shown to last either for similar or longer duration[1,47] than the time that rats exhibit pain-related behaviors after laparotomy performed under inhalant anesthetics.[49,50] However, the efficacy of additional analgesics in postoperative recovery of rats subjected to surgery under this balanced anesthetic mixture is unknown. Interestingly, in rats undergoing abdominal surgery under a ketamine–xylazine anesthetic combination, administration of the analgesic buprenorphine, butorphanol, or ketoprofen did not have any beneficial effect over that associated with subcutaneous dextrose solution during postoperative recovery.[52] In addition, preanesthetic administration of buprenorphine in combination with ketamine–medetomidine anesthesia is known to cause high mortality in rats.[51]

In the rat, pain or distress leads to body weight (BW) loss due to reduced food and water intake.[10,15,35] The current studies were designed to evaluate the effect of the balanced anesthetic mixture on postsurgical recovery, as measured by daily changes in BW and water intake, of rats subjected to laparotomy in the presence or absence of a single postoperative dose of the analgesic ketorolac.[6] The results were compared with those of housing and anesthesia controls as well as of laparotomized rats receiving a single serving of fruit-flavored sugared cereal

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Table 1. Experimental groups and treatment conditions

Group	Body weight (g) on day of surgery (day 0)	Description
HC	374 ± 10.1	Housing control: rats with no other manipulation.
AC	376 ± 9.0	Anesthesia control: anesthetized rats with no surgery.
LT	376 ± 8.6	Laparotomy + treat: laparotomized rats provided with a single 4- to 5-g serving of fruit-flavored sugared cereal on day 0.
LV	375 ± 8.0	Laparotomy + vehicle: laparotomized rats that received 0.9% saline intramuscularly at 60 min postanesthesia.
LK	375 ± 8.0	Laparotomy + ketorolac: laparotomized rats that received 5.0 mg/kg ketorolac intramuscularly at 60 min postanesthesia.

Values are presented as mean ± SE (n = 10 per group).

as a form of nonpharmacologic management of postoperative pain.[65] An initial experiment (Experiment 1) was carried out to establish the appropriate time point for the administration of the analgesic, which was determined by evaluating the return of pedal withdrawal reflex (PWR) after exposure to the anesthetic mixture.

Materials and Methods

Animals. The data in this manuscript are generated from 2 different experiments performed in pathogen-free adult (350 to 450 g) male rats (*Rattus norvegicus*). In Experiment 1, the time course of effects of the anesthetic mixture was evaluated in Long–Evans (HsdBlu:LE) rats (Harlan Sprague-Dawley, Indianapolis, IN). In Experiment 2, the effects of the analgesic ketorolac and of fruit-flavored sugared cereal on postoperative recovery after ketamine–xylazine–acepromazine anesthesia were evaluated in Sprague–Dawley (Crl:SD) rats (Charles River, Portage, MI).

Upon arrival at the Emory vivarium, the rats were maintained under standard rat husbandry conditions. They were pair-housed in polycarbonate solid-bottom cages (floor area, 930 cm^2) with stainless steel grid tops and containing ground corncob bedding (Bed-o'cobs, The Andersons, Maumee, OH). They were maintained on a 12:12-h light:dark cycle and received pelleted chow (Laboratory Rodent Diet 5001, St Louis, MO) ad libitum and tap water by bottle. The rats were allowed to habituate to the new environment for 1 wk before being used in experiments. All procedures were approved by the Emory University Institutional Animal Care and Use Committee in compliance with National Institutes of Health recommendations based on National Research Council guidelines.[41]

Anesthetic. The balanced anesthetic solution was prepared as described previously[56] by mixing 2.5 ml ketamine hydrochloride (Ketaset, 100 mg ketamine/ml, Wyeth/Fort Dodge Animal Health, Overland Park, KS), 2.5 ml xylazine hydrochloride (Rompun, 20 mg xylazine/ml, Bayer Animal Health, Shawnee Mission, KS), 1.0 ml acepromazine maleate (10 mg acepromazine/ml, Vedco, St Joseph, MO) and 4 ml sterile water. The composition is the same as that used in neuroendocrinologic studies by other investigators.[46] The suggested dose of this anesthetic mixture (ketamine:xylazine:acepromazine, 25:5:1 mg/ml) for simple to moderate surgeries is 125/100 g BW (ketamine:xylazine:acepromazine, 31.25:6.25:1.25 mg/kg BW) and 150 µl/100 g BW (ketamine:xylazine:acepromazine, 37.5:7.5:1.5 mg/kg BW) for extensive surgical procedures.[56] In the current studies, laparotomy was considered a moderate surgical procedure, and therefore the mixture was administered subcutaneously at a dose of 125 µl/100 g BW with a 25-gauge needle. For subcutaneous administration of the anesthetic mixture, the rat was held gently under a white cloth towel, and the skin behind the nape on the back was lifted between the thumb and forefinger of the experimenter's left hand. Using the right hand, the experimenter carefully injected the solution at the base of the skin fold so that anesthetic mixture remained in the subcutaneous space.[56] The technique was similar to that described by Nebendahl,[42] except that the injection was made from behind, and the head of the rat was inside the towel.

Analgesic. A 20 mg/ml solution of ketorolac tromethamine (Novaplus, Abbott Laboratories, North Chicago, IL) was prepared by diluting the 30 mg/ml pharmaceutical preparation (obtained from Emory University Hospital pharmacy) with sterile 0.9% saline. At 60 min after administration of the balanced anesthetic mixture, a single dose (5 mg/kg, 25 µl/100 g BW) of the ketorolac solution was administered into the right quadriceps muscle while the rats were still under anesthesia (30 to 40 min after surgery) and recovering on a temperature-regulated heating pad. This dose of ketorolac was based on previous studies.[1,17,22,29]

Ketorolac and vehicle solutions were provided in coded bottles by the University Veterinary staff, and investigators were blinded to the treatments. The code was broken after completion of data collection and analysis.

Fruit-flavored sugared cereal. A one-time supplement of sweetened fruit-flavored cereal (Fruit-Loops, Kellogg, Battle Creek, MI) was used as a treat in the current study. The cereal has also been used to train rats in a radial arm maze behavioral task.[16] In the current study, 4 to 5 g cereal was placed near approximately 10 food pellets on the cage floor. After laparotomy, designated rats were transferred into these cages. Cereal intake was not recorded, and it is not known over what period the rats consumed the cereal. The supplement was provided only once, to avoid development of anticipatory behavior.[40]

Experimental design. *Experiment 1*. This experiment was designed to monitor intraoperative effects of the anesthetic mixture and to establish the time for administration of the analgesic. This aim was carried out in an experimental design requiring stereotaxic surgery. Because of limited stereotaxic instrumentation, there was considerable lag in the surgical preparation of one rat to another, which made it possible to record the progression of anesthetic effects accurately. After administration of the anesthetic mixture, the time points for the onset of specific indices of anesthesia and analgesia were recorded.[9,14] These indices were: 1) loss of righting reflex, 2) development of immobility with retention of PWR, 3) immobility and loss of PWR, 4) immobility with return of PWR, 5) return of righting reflex, and 6) return of postural stability. The PWR was monitored as withdrawal from the standard toe-pinch applied using a mosquito-type hemostat.[9]

***Experiment 2*.** This experiment was designed to evaluate the effects of the different treatments on postoperative recovery in rats subjected to bilateral laparotomy using the anesthetic mixture. The surgical model was similar to that used by other investigators,[34,35,52] but in the current study, bilateral laparotomy was performed. Body weight was recorded, and rats were assigned to 1 of 5 treatment groups (Table 1). After recovery

Table 2. Progression of anesthetic effects

Stage	Measure	Time after anesthesia (min)
1	Loss of righting reflex	3.4 ± 0.4
2	Immobile, with pedal withdrawal reflex	6.1 ± 0.5
3	Immobile, no pedal withdrawal reflex	9.6 ± 0.7
4	Immobile, pedal withdrawal reflex present again	83.3 ± 6.3
5	Return of righting reflex	122.3 ± 4.1
6	Righting reflex present again, with postural stability	155.1 ± 7.3

After induction of ketamine–xylazine–acepromazine anesthesia at 0 min, we performed the toe-pinch test during stages 2, 3, and 4. During stages 2 and 3, toe-pinch was applied every 3 to 5 min to alternating hind legs, and then every 7 to 10 min thereafter. Stage 5 was characterized by the rat regaining the ability to move all 4 legs. Regaining the ability to stand characterized stage 6 and was recorded in the rats' home cages. Values are presented as mean ± SE (n = 10 per group).

from surgery, all rats were housed singly in clean cages with fresh bedding and a few food pellets placed in a corner of the cage. Housing-control (HC, unmanipulated but singly housed) and anesthesia-control (AC, anesthetized but not subjected to laparotomy) groups were included to evaluate the influence of housing and anesthesia alone on the measured variables. While on a temperature-regulated heating pad, the laparotomy-vehicle (LV) and laparotomy-ketorolac (LK) groups received either vehicle (0.9% saline) or analgesic, respectively, intramuscularly at 60 min after administration of the anesthetic mixture. The laparotomy-treat (LT) group received 4 to 5 g of a fruit-flavored sugared cereal placed near the food pellets inside the cage. Body weight and water intake were recorded daily at 1200 for 4 consecutive days. The day of surgery was designated as day 0. The experiment was terminated on day 4 because previous studies have shown that rats have reestablished their circadian rhythm of HPA axis activity by this day.[56]

Surgical procedures. ***General.*** Surgeries were performed aseptically with rats under sound anesthesia (loss of PWR). To reduce blood loss, atraumatic (noncutting) surgical techniques were followed, and the use of surgical blades was avoided. Nonabsorbable suture materials were used because rats were euthanized within 2 wk after surgery. Instruments were decontaminated and disinfected between uses by keeping them immersed in 50% to 70% ethanol,[25] and the ethanol was drained off before any instrument was used. The ethanol was changed after every 5 surgeries. To reduce contamination, different instruments were used for each step of the surgical process; this practice allowed sufficient time for the instruments to remain in the ethanol bath. The technique for the maintenance of sterility and asepsis in the current study was the same as that used routinely in our laboratory to surgically prepare multiple rats for neuroendocrinologic studies.[2,33,56-58,63] These studies have demonstrated that by day 4 after surgery, these rats exhibit normal basal plasma corticosterone, normal circadian corticosterone patterns,[2,33,56-58,63] and a normal corticosterone fast feedback-induced suppression of the HPA axis reactivity to stress,[57] which are all affected by trauma and infection.[46]

On the day of surgery (day 0), the rats were weighed and anesthetized with the anesthetic mixture. When rats were immobile, the incision areas were shaved gently and cleaned thoroughly with 70% ethanol. At the end of surgery, povidone–iodine solution was applied around the incisions, and the rats were allowed to regain mobility on a temperature-regulated heating pad. Periodically the rats were turned over for uniform heating and to evaluate the recovery status,[56] but the body temperature was not recorded. When rats began to exhibit the righting reflex (Experiment 2), they were transferred to polycarbonate cages and housed singly under standard rat husbandry conditions. All surgeries were completed before 1200.

Stereotaxic surgery. The stereotaxic surgical procedure used in the current study is considered in the minor-to-moderate category.[20] Immediately after rats were immobile with loss of PWR, they were placed on a temperature-regulated heating pad and mounted onto a stereotaxic frame with blunt ear bars (David Kopf Instruments, Tujunga, CA), with the incisor bar placed at 3.3 mm below the interaural line (horizontal zero). Pain reflexes were monitored (Table 2) using a standard toe-pinch technique with a mosquito-type hemostat. The skin on the top of the skull was wiped thoroughly with 70% alcohol and was cut (about 2 cm) sagitally along the midline by using a scalpel blade. The skin was pulled apart, and the cranium was exposed. After the surface was cleaned free of tissue, a burr hole (outer diameter, 0.5 to 0.75 mm) was made to approach the nucleus accumbens by using coordinates (2.0 mm anterior to bregma, 1.5 mm lateral to midline) derived from a standard rat brain atlas.[43] Thereafter, a 26-gauge stainless steel guide cannula (Plastics One, Roanoke, VA) was lowered at 7.0 mm beneath the surface of the skull and secured with screws and dental cement (Plastics One). After the cement had hardened, the guide cannula was closed with a stylette, and the skin at each end of the incision was tied together with sterile surgical suture (black braided 2-0 silk, Ethicon, Cornelia, GA). The rat then was removed from the stereotaxic frame and allowed to recover mobility over a temperature-regulated heating pad during which period the PWR was monitored.

The average length of surgery was approximately 30 to 40 min and if the PWR returned before the surgery was completed (2 rats), additional anesthetic was administered (25 µl/100 g BW). In the current study, data from these 2 rats were excluded. The average BW of the rats used in this study was 401 ± 6.9 g (n = 12). All 12 rats were surgically prepared on 3 successive days (4 rats/d).

Bilateral laparotomy. Immediately after rats were immobile with loss of PWR, a 2-cm skin incision at the base of the lumbar vertebrae and posterior to the rib cage in the dorsal flank region was made with scissors. The retroperitoneal space then was approached through a small incision, made with iris scissors, in the exposed abdominal tissue. Once the cavity was visible, the blades of the iris scissors were opened to stretch the muscle and widen the hole to approximately 1.5 cm. Singley forceps then were lowered into the retroperitoneal space and organs were probed by nudging. Thereafter, the abdominal tissue was closed with nonabsorbable sterile surgical suture (black braided 2-0 silk, Ethicon); the extra length of suture was cut close to the knot to avoid obtruding the skin incision. Incisions in the skin were closed with wound clips. The rat was turned over, and the surgical procedure was repeated on the other side. The average length of surgery was 10 min. Four investigators participated in the performance of the surgical procedure on these rats: 1 investigator prepared the rats for surgery; another performed the bilateral laparotomy (30 rats); a 3rd investigator carried out

Table 3. Body weight change and water intake during the acute postoperative phase (1st 24 h after surgery)

	Body weight loss		Water intake (ml)	
Group	net (g)	% of day 0 value	total	per 100 g
Housing control	3.2 ± 1.05	0.9 ± 0.28	50 ± 2.5	13.4 ± 0.70
Anesthesia control	9.3 ± 2.02[a]	2.5 ± 0.56	45 ± 2.0	12.2 ± 0.49
Laparotomy + treat	8.2 ± 1.94[a]	2.2 ± 0.51	46 ± 2.3	13.8 ± 0.72
Laparotomy + vehicle	11.6 ± 1.54[b]	3.1 ± 0.41	52 ± 4.2	12.7 ± 0.60
Laparotomy + ketorolac	8.8 ± 1.03[a]	2.3 ± 0.25	51 ± 2.8	14.0 ± 1.05

Values are presented as means ± SE (n = 10 per group).
[a]$P < 0.05$ versus value for housing control.
[b]$P < 0.01$ versus value for housing control.

suturing and so on; and the remaining investigator followed the rats through recovery. Surgery was completed by 1200, and all rats exhibited the return of righting reflex by 1400.

Pain indices. Parameters related to growth such as changes in BW and intake of food and water are considered as general measures of pain or distress.[9,10,13,15,34,35,52] In the current study, BW was measured before surgery and every day thereafter at 1200. In addition, the rats were observed for visible signs of species-typical pain and distress[9,10,20] while residing in their cages, before being removed for weighing. However, the incision sites were not manipulated.

In addition, 24-h water intake was monitored. On day 0, water bottles were filled and capped with rubber stoppers with sipper tubes, and the flow was checked. Before a bottle was placed on the cage lid, the total weight was recorded. Every 24 h, the bottles were weighed, and differences in weight from the previous day were considered as the amount (g) of water consumed. Bottles were handled carefully to avoid spillage. This process was repeated until day 4 without refilling of the bottles. A 1-g decrease in bottle weight was equated to a 1.00-ml water intake.

Data analysis. The results from Experiment 2 were evaluated in 2 categories, acute phase (the first 24 h after surgery) and recovery phase (days 1 through 4). During the acute phase, changes in BW and water intake were evaluated across treatment conditions by 1-way analysis of variance (ANOVA).

During the recovery phase, the effect of treatment conditions on changes in BW and water intake were evaluated by 1-way ANOVA corrected for repeated measures across days,[64] and when overall treatment effect or interaction was significant, the level of significance between treatment conditions on each day was tested by computing F values for the simple main effects.[64] The significance of differences in BW changes from day 0 was computed for each group separately after repeated measures ANOVA by the Student–Newman–Keuls test when the treatment effect or interaction was significant, otherwise by the Tukey test using SPSS statistical software (SPSS, Chicago, IL). Unless otherwise indicated, values are presented as mean ± standard error and are based on 10 rats per group. The null hypothesis was rejected for all values of $P \leq 0.05$.

Results

Experiment 1–intraoperative effects. The progression of anesthetic effects is shown in Table 2. PWR was absent from 10 ± 0.7 min until 83 ± 6.3 min after administration of the anesthetics, thus providing sufficient time to conduct many routine moderate surgical procedures. The return of righting reflex (stage 5, Table 2) was observed by 122.3 ± 4.1 min, but the rats regained righting reflex with postural stability (stage 6, Table 2) by 155 ± 7.3 min. Overall, the anesthetic mixture was both fast- and long-acting.

Experiment 2. *Signs of pain or distress.* In Experiment 2, rats exhibited the return of righting reflex (similar to stage 5, Table 2) by 120 min postsurgery, when the rats were transferred into their cages. Before 'lights-off,' all rats were alert to the presence of the investigator (about 5 to 8 h postsurgery). Thereafter, the effects of treatment on visible signs of pain or distress[9,10,20] were recorded every 24 h when the rats were handled for weighing. However, the incisions were not manipulated. By 24 h after surgery, all rats exhibited rearing behavior, irrespective of treatment condition. Moreover, none of the rats vocalized, showed porphyrin staining around the eyes and nose, or behaved aggressively when removed from the cage. Piloerection was also not evident, which suggested normal grooming behaviors. In addition, none of the rats exhibited signs of selfmutilation of the subcutaneous injection site. Similarly, the LK rats did not selfmutilate the right quadriceps muscle area. This pattern of behavior was similar on other days, suggesting successful recovery. By day 4, deposition of connective tissue was noticeable at the incision site, suggesting a normal wound healing process.

Acute phase. The effects of anesthesia and postoperative treatments on BW and water intake during the first 24 h after surgery (acute phase) are shown in Table 3. As can be seen, all rats lost BW (maximum loss, 6%) during this period, irrespective of treatment condition, with the HC group showing the smallest loss of BW. The difference in BW change between HC and other groups was significant ($P < 0.05$). Interestingly, the loss in BW in the AC group was similar to that of rats subjected to laparotomy (LV, LT, and LK). The differences in BW were not due to water intake, as that did not significantly differ between treatment groups (Table 3).

Recovery phase. The effects of anesthesia and treatments after laparotomy on postoperative recovery in BW are shown in Figure 1. Overall, there was a significant treatment effect ($F_{4, 45} = 7.58$, $P < 0.01$), day effect ($F_{4, 180} = 64.20$, $P < 0.01$), and treatment × day interaction ($F_{16, 180} = 3.22$, $P < 0.01$). By day 4, all rats had recovered their initial BW. Importantly, BW in the LT group was not different between days 0 and 3, and it was significantly higher on day 4 ($P < 0.05$) compared to day 0, suggesting an accelerated growth pattern. BW in the LK group did not significantly differ between days 0 and 3,or between days 0 and 4. It should be noted that ANOVA performed without the HC group yielded neither a treatment effect ($F_{3, 36} = 1.00$) nor an interaction between treatment × day ($F_{12, 144} = 0.92$) but only a significant effect of day ($F_{4, 144} = 41.7$, $P < 0.01$).

The effects of anesthesia and treatments after laparotomy on water intake are shown in Figure 2. Overall, there was no significant treatment effect ($F_{4, 45} = 0.26$) or treatment × day interaction ($F_{12, 135} = 1.67$). Accordingly, no post hoc analysis was performed. However, there was a significant day effect ($F_{3, 135} = 7.20$, $P < 0.01$), which could be attributed to a general decrease in water intake on day 2. However, the differences in the water intake between days 1 and 2 were not significant for any treatment group, as established by Tukey tests.

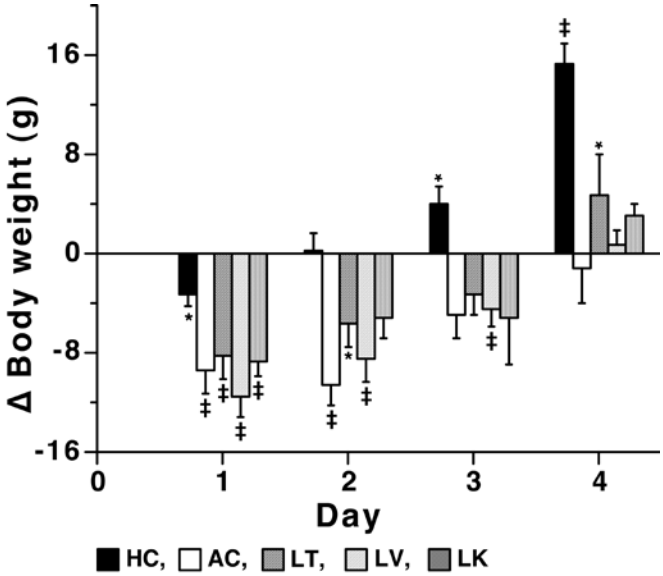

Figure 1. The effect of anesthesia and postoperative treatments on body weight recovery in rats from Experiment 2. Day 1 data are the same as in Table 3. Values are presented as mean ± standard error (n = 10 per group). *, $P < 0.05$ and ‡, $P < 0.01$ versus value for day 0. Group abbreviations are as defined in Table 1. The change in body weight from that on day 0 is represented on the y axis.

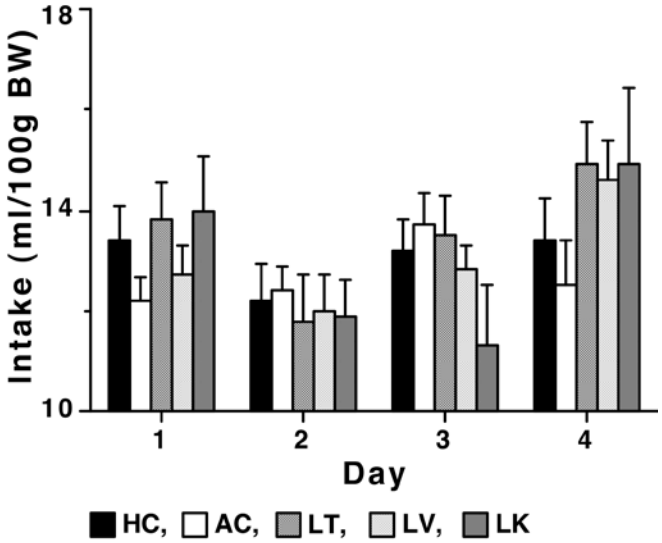

Figure 2. The effect of anesthesia and postoperative treatments on water intake per unit body weight in a 24-h period in Experiment 2. Day 1 data are the same as those in Table 3. Values are presented as mean ± standard error (n = 10 per group). Group abbreviations are as defined in Table 1.

Discussion

The results suggest that 1) the ketamine–xylazine–acepromazine anesthetic mixture is fast- and long-acting and provides sufficient immobility, loss of consciousness, and analgesia of rats; 2) during the acute phase, rats in the laparotomy groups did not lose more BW than rats exposed to anesthesia alone; 3) water intake during both the acute and recovery phases did not significantly differ between treatment groups; 4) postsurgical ketorolac administration did not minimize BW loss during the acute phase nor cause any adverse effects under this anesthetic regimen, and 5) a single provision of a sugared treat had a salutary effect on BW recovery.

Balanced anesthesia takes advantage of the different beneficial effects of several drug classes. Its objectives are to induce immobility, loss of consciousness, muscle relaxation, and analgesia by using a combination of drugs with specific individual properties and to reduce the potential adverse effects associated with individual agents.[27,31] In addition, each drug can potentiate the effects of other drugs; thus the amount of the individual components needed in the anesthetic mixture is less than those required when these drugs are administered alone.[1] Therefore the combination of the 3 drugs used in the present study is expected to provide balanced surgical anesthesia in rodents.

Ketamine is a noncompetitive N-methyl-D-aspartate glutamate receptor antagonist that produces anesthesia by inducing dissociation of sensory, motor, integrative, memory, and emotional activities in the brain, leading to catalepsy without central nervous system depression.[11,12] Interestingly, administration of a subanesthetic dose of ketamine during surgery was shown to reduce postoperative analgesic requirements in humans,[19] and in rats the analgesic properties were shown to last for more than 8 h.[47] Ketamine also suppressed c-fos expression in dorsal horn neurons after acute constrictive sciatic nerve injury in the rat.[24] An ameliorative effect of ketamine on behavioral despair in rats has also been reported.[67] However, ketamine is a poor muscle relaxant[18] and therefore it is used in combination with other anesthetics such as xylazine, an alpha-2 adrenergic agonist with sedative and analgesic properties.[1,12,18] The 3rd component of the anesthetic mixture, acepromazine, is a phenothiazine derivative with sedative, muscle-relaxant, and antiemetic properties. It is not an analgesic by itself[1] but can potentiate the analgesic effects of other compounds. Acepromazine is often used in combination with ketamine, where it has been shown to counteract muscle rigidity. Therefore the combination of these 3 drugs potentially blocks nociceptive inputs during minor to moderate surgery as well as during the postsurgical recovery period, when analgesics are deemed important.

The use of a ketamine–xylazine–acepromazine combination for anesthesia has been described in mice,[3] rats[33,34,39,46,56-58] and rabbits.[37] In the current study, all rats receiving either 125 µl/100 g BW or 150 µl/100 g BW of the balanced anesthesia mixture survived (n = 62). Other studies in which the combination of anesthetics was administered have used different ratios and concentrations of the 3 compounds, with ratios varying from 20:2:1,[34] 30:3:1,[8] 50:5:1,[32] 51:1:1,[44] to 80:8:1.[54] Thus, there is a great margin of safety with the use of this anesthetic mixture, and the dose can be adjusted depending on the length of surgery.

Ketorolac is an injectable nonsteroidal anti-inflammatory drug with antipyretic and analgesic properties that is effective for moderate to severe postoperative pain in animals as well as in humans.[6,17,38,48] In rats, it has been shown to have an analgesic effect[23,30] and to cause less pain-on-injection and postinjection than do other nonsteroidal anti-inflammatory drugs.[7]

The time point at which the analgesic was administered was based on Experiment 1 as well as on the report of Sharp and colleagues.[52] In Experiment 1, the PWR returned at approximately 83 min (Table 2) after injection of the anesthetic mixture; therefore the analgesic was given 60 min after anesthetic administration to allow enough time for the drug to distribute in the body and to begin its action. When using ketamine–xylazine anesthesia, Sharp and colleagues[52] administered analgesics at 30 to 45 min after initiation of the surgical procedure.

At present, there are no established guidelines to evaluate pain-related behaviors in rats anesthetized using injectable anesthetics and subjected to surgical manipulations. Behavioral effects are predominant only during the first 2 to 3 h after

surgery.[49,50] In the current study, all surgery was completed by 1200, and the rats exhibited the return of righting reflex (Table 2) after only 2 h. Thereafter, they were asleep, possibly due to the sedative effects of the drugs or sleep time or both. Therefore, in this study, it was not possible to measure behavior during the initial 2 to 3 h postsurgery. Evaluation of differences in the pain threshold by using the hot-plate test[2] and evaluation of ultrasonic vocalizations[21,61] may be ideal when using injectable anesthetics. However, in the current study, the rats were intended for other experiments, and these tests were not performed.

In Experiment 1, 2 rats that underwent the stereotaxic surgery required supplemental anesthetics. It is possible that during subcutaneous administration, some quantity of the anesthetic was deposited intradermally. Generally, rats that are not steady during administration of the anesthetics often encounter early return of the PWR with and without a bleb close to the injection site. In addition, excessive urination during prolonged surgical procedures can trigger early return of the PWR.

In Experiment 2, the HC group lost BW during the acute phase (Table 3). However, by day 2, these rats had regained their BW and thereafter exhibited a steady increase in weight (Figure 1). Thus, housing the rats singly contributed to the net loss in BW in the AC and laparotomy groups. In addition, anesthesia alone adversely affected BW recovery. Other investigators[34,35,49,50] have reported similar BW loss with both inhalant and injectable anesthetics. However, in the current study water intake was not significantly affected in the anesthesia alone rats. It is possible that in the absence of surgical stimulation, isolation housing and the suppressive effect of ketamine on glucocorticoid tone[59] contributed to altered energy balance and thus BW recovery in the AC group. It would be of interest to determine whether these effects could be reversed with sugared cereal supplements.

Interestingly, the BW loss in the laparotomy groups was similar to that of the anesthesia-alone group. This finding is in sharp contrast to studies in which laparotomy was performed under inhalant anesthetics, where rats undergoing surgery lost more BW than did rats subjected to anesthesia alone.[34,35] In such studies, postoperative analgesics decreased BW loss.[34,35] However, in the current study, 5 mg/kg ketorolac did not reduce weight loss during the acute phase and did not exhibit any additional beneficial effect on BW recovery. A similar lack of effect of other nonsteroidal anti-inflammatory drugs has been reported to occur in rats when surgery is performed using ketamine–xylazine anesthesia.[52] A higher dose of ketorolac was not tested because it has been reported to interact adversely with gentamicin,[29] an antibiotic that is used routinely in our laboratory as a catheter-lock solution.[56] In addition, an oral dose of 3.2 mg/kg ketorolac produced sufficient plasma concentrations to provide analgesic effects.[17]

Of the rats that underwent laparotomy, only the LT group exhibited a significant increase in body weight on day 4 (Figure 1) relative to their initial BW. This finding suggests a moderate beneficial effect of sugared cereal supplementation during the post-operative recovery period. Indeed, other relatively simple measures such as environmental enrichment, novelty, and social support have been shown to improve the postoperative wellbeing of animals.[65] Another study reported that rats on energy supplementation (5% dextrose) alone gained more BW postoperatively than did those receiving energy supplementation in combination with analgesics.[52]

In the current study, Experiment 1 was done in Long–Evans rats and Experiment 2 was carried out in Sprague–Dawley rats. However, whereas the induction of anesthesia and recovery were similar in the 2 strains, it is not known whether the analgesic effects are similar also. Due to the short survival duration of rats in Experiment 1, BW and water intake were not recorded. In addition, sex differences in the potential analgesic effects of the anesthetic mixture were not investigated.

Overall, these studies suggest that postsurgical BW loss in rats after use of a ketamine–xylazine–acepromazine anesthetic mixture is primarily due to non-pain-related distress and that this effect may have masked the potential beneficial effects of ketorolac. In addition, our findings show that the requirement for postoperative analgesia is influenced by many factors, including choice of anesthetic, surgical technique, and postoperative environment. Furthermore, our data suggest that the compatibility and effectiveness of drugs should be evaluated for the given condition before guidelines for their use are established.

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