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Effect of a Short-term Fast on Ketamine–Xylazine Anesthesia in Rats

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Although ketamine–xylazine (KX) anesthesia is commonly used in rats, it is often reported to have an inconsistent anesthetic effect, with a prolonged induction time, an inadequate anesthetic plane, or a very short sleep time. Blood flow to the liver is known to shift after a meal in rats, perhaps explaining anesthetic variability among rats with variable prandial status. The current study tested the hypothesis that a short period of fasting (3 h) prior to induction with intraperitoneal KX anesthesia would provide a shorter time to recumbency, a longer total sleep time, and a more consistent loss of toe pinch response than would fed rats. Two groups of male Sprague–Dawley rats were used in blinded, crossover experiments. KX anesthesia was administered at 2 different doses (50 mg/kg–5 mg/kg and 70 mg/kg–7 mg/kg) after ad libitum feeding or a 3-h fast. There were no significant differences between groups in induction time, total sleep time, or loss of toe pinch response. We conclude that fasting rats for 3 h prior to KX intraperitoneal anesthesia does not affect induction time, total sleep time, loss of toe pinch response or reduce KX anesthetic variability in male Sprague–Dawley rats.

Abbreviation: KX, ketamine–xylazine.

Ketamine is a noncompetitive *N*-methyl *D*-aspartate receptor antagonist that provides amnesia, analgesia, dissociation from the environment, and immobility.[4,17] Ketamine, combined with other agents, is a commonly used injectable anesthetic in rats, mice, and other rodents.[7] Ketamine is often combined with xylazine, an α2 adrenergic agonist, which supplements ketamine's effect with analgesic properties, muscle relaxation, and sedation. The combination of ketamine–xylazine (KX) provides a relatively safe anesthesia that can be administered without the need for specialized equipment.[7] Typically KX is administered to rats as a single intraperitoneal injection. Although used commonly, individual rats can have highly variable responses to KX anesthesia [6, 10,11,19] with some rats not achieving an acceptable surgical anesthetic plane.[11,23]

Ketamine, the general anesthetic component of KX anesthesia, has a rapid onset of action and high lipid solubility. Initially, ketamine is distributed to highly perfused tissues, such as the brain.[21] Ketamine has a high hepatic clearance (1 L/min) and a large volume of distribution (3 L/kg), resulting in an elimination half-life of 2 h.[24] The high hepatic extraction ratio (0.9) suggests that an alteration in hepatic blood flow could influence ketamine's clearance rate.[21] This characteristic means that the limiting factor in ketamine metabolism is the hepatic blood supply's ability to deliver drug to hepatocytes and not the enzymatic ability of the hepatocytes to metabolize the drug.[13] Xylazine has a large volume of distribution after administration and a rapid metabolic clearance that is not primarily dependant on hepatic blood flow.[9] Hepatic blood flow is influenced by the fasting–fed state. Thirty minutes after a meal, splanchnic and hepatic blood flow increase in rats.[1,18] In addition, withholding food has been shown to decrease plasma clearance of several drugs.[5] A lack of uniform prandial status among a group of rats could be a compounding cause of anesthetic variability.

Due to their high metabolic rate and their inability to vomit, rats are not fasted routinely prior to anesthetic events. The amount of food recently consumed and the interval between the most recent feeding and induction of anesthesia is usually unknown. Investigators typically control for reasons for rats to react differently to the same dose of anesthesia. Age, sex, genetic, and environmental factors, as well as inherent interindividual variability, all contribute to anesthetic variability. To our knowledge, the importance of short-term fasting relative to variability in response to KX anesthesia in rats has not been studied previously.

The objective of this study was to determine whether a short fast before an anesthetic event would lead to faster induction and longer, more consistent anesthesia in fasted rats compared with their fed cohorts.

Materials and Methods

Male Sprague–Dawley (Crl:SD) rats [weight, 350 to 440 g (experiment 1); 430 to 560 g (experiment 2)] were chosen to eliminate the possible cyclical reproductive variability in female rats and because they are a popular stock, with applications in many areas of research. Animals were housed in the AAALAC-accredited animal facilities at the University of Florida. All procedures were in compliance with the *Guide for the Care and Use of Laboratory Animals*[12] and were included in a protocol approved by the University of Florida IACUC. Rats were acclimated to a single-housing environment for at least 2 wk and were handled for 5 min daily for 5 d each week prior to the start of the study to acclimate them to human contact. All animals were antibody-negative for coronavirus (sialodacryoadenitis virus, rat coronavirus), Kilham rat virus, lymphocytic choriomeningitis virus, mouse adenovirus, *Mycoplasma pulmonis*, pneumonia virus of mice, rat minute virus, rat parvovirus, reovirus type 3, Sendai virus, Theiler murine encephalomyelitis virus, and Toolan H1 virus. In addition, the rats were free of external and internal parasites. Animals were housed on corncob bedding (Harlan, Madison, WI) in a temperature-controlled room

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(21 to 2 °C) in polycarbonate microisolation caging on a ventilated rack (Allentown, Allentown, NJ). Standard rodent diet (Teklad 8460 standard rodent diet, Harlan Teklad) and purified reverse-osmosis water were offered ad libitum except during fasting, when food was removed. Prestudy food consumption data were recorded after acclimation and before the experiments were started to determine the rats' food intake during a period that corresponded to the 3-h preanesthetic period.

Experimental design. Two crossover experiments were performed, each using a different dose of KX anesthesia (Ketaset, Fort Dodge, Fort Dodge, IA; Anased, Lloyd Laboratories, Shenandoah, IA). Experiment 1 used 50 mg/kg ketamine, 5 mg/kg xylazine, and 20 rats; experiment 2 used 70 mg/kg ketamine, 7 mg/kg xylazine, and 10 rats. Doses were chosen to represent the low- to midrange published KX dosages. These doses were expected to induce a surgical plane of anesthesia of short to medium duration. The crossover was between fed and fasted groups within each experiment. In each experiment, rats were divided randomly into 2 treatment groups, a fasted group and a fed group, and all underwent intraperitoneal anesthetic induction with KX after treatment. After a 14-d washout period, anesthetic induction was repeated, with rats previously included in the fed group now included in the fasted group and vice versa. Thus, within each experiment, each rat served as its own control. Previous anesthesia studies have used shorter washout periods[2] (1 wk or less), but we used a 14-d washout period to further reduce the possibility of a carryover effect. There was an interval of 6 wk between the 2 experiments. Data from each experiment were analyzed separately.

Rats were housed on a 12:12-h reverse light:dark cycle (lights off, 1100; lights on, 2300). The reverse light cycle was used to facilitate the fed and fasted treatment groups without prolonged fasting of the rats. Fed rats retained their normal, ad libitum feeding schedules. Fasted rats underwent a 3-h preanesthetic fast. No rat was fasted for longer than 5 h (including anesthesia time). The investigator was blinded to group assignments until the end of each study. At 1 h prior to start of the dark cycle, food was removed from rats in the fasted group. The fed group had access to food ad libitum until anesthesia was administered (2 h into the dark cycle), and both groups of rats were returned to cages after recovery with ad libitum access to food. For the fed group, food was weighed at 1 h prior to the dark cycle and again just before injection. At 2 h after the start of the dark cycle, the rats were transported to a separate procedure room for anesthesia. We anesthetized 3 or 4 rats daily until all rats received the first randomized treatment (fed or fasted) and then again after the washout period.

Rats were placed on a heated surface, and rectal temperatures were recorded throughout the anesthetic period by using a digital thermometer (Procter and Gamble, Cincinnati, OH). All rats remained normothermic throughout the anesthetic period. Beginning at 1 min after injection, rats were rolled onto their sides once every minute to check for loss of the righting reflex. When a rat was not able to right itself (loss of righting reflex), the time period between injection and loss of righting reflex was recorded as time to recumbency. One minute after time to recumbency was recorded, a toe pinch was applied to each rat to determine whether the withdrawal reflex was present. The toe-pinch response test was conducted using a 0.75-in. binder clip (item no. 825182, Office Depot, Boca Raton, FL) applied to the areas of metatarsals or metacarpals. The clip provided enough pressure to cause a withdrawal reflex in an awake or a lightly anesthetized animal but did not cause injury to the rats. This process was continued once each minute, with one pinch to a hindlimb and one pinch to a forelimb. The length of time that each rat's toe-pinch response remained negative was recorded for hindlimbs and forelimbs. Rats were observed until they first returned to a sternal position. When a rat was able to perform 3 consecutive rightings, the time was recorded as return of righting reflex. Total sleep time was considered to be the time elapsed between loss and return of the righting reflex.

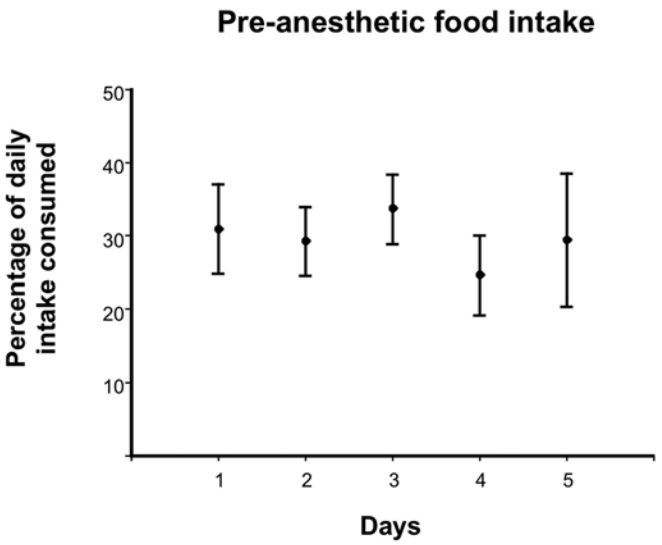

Figure 1. Median food intake of all rats during a 3-h preanesthetic period as measured for 5 d before the start of the experiments.

Data analysis. All analyses were performed in SAS (version 9.1, SAS Institute, Cary, NC). Model diagnosis was conducted, and residual analysis showed that the Gaussian distribution assumption was satisfied. Two subjects in the fasted group of experiment 1 were excluded due to experimental error. Linear regression models were performed for each outcome variable (excluding toe pinch response in hindlimbs) to determine whether carryover (effect of previous treatment), period (time period regardless of treatment assignment), or treatment (fasting) effects were present. The data for the toe-pinch response in the hindlimbs were not analyzed in this experiment due to the high number of animals (fed, n = 9; fasted, n = 13) that had 0 for this variable. As for experiment 1, data analysis for experiment 2 was performed by using a linear regression model for each outcome variable to determine whether carryover, period, or fasting effects were present. For the time-to-recumbency variable in both experiments, any rat that did not become anesthetized (that is, time to recumbency was 0) was excluded from the analysis, because a 0 for that variable essentially became an infinite value and did not fit the model. This exclusion applied to 7 rats in experiment 1 and to 1 rat in experiment 2.

In addition to the standard statistical analysis, we assessed differences between fasted and fed rats among only the rats that became anesthetized. This process was to ensure the lack of significant effect between the fed and fasted treatment groups in each experiment, among only the rats that became anesthetized. To perform this exploratory analysis, we eliminated, for each variable, any rat that did not respond (had a 0 value) for that particular variable and then repeated the regression analysis.

Because this study was an initial investigation, we did not have estimates required to examine power and sample size prior to the study. The power analysis after the study enabled us to assess the magnitude of the difference we would have been able to detect, according to our estimates of variance. Analysis was based on a 2-sided paired t test with level of significance of 0.05 and of 0.80 for power.

Table 1. Comparison between fed and fasted KX-anesthetized rats

Experiment		*n*	Median	Mean	1 SD	Minimum	Maximum	*P* carryover	*P* treatment
				Time to recumbency (min)					
1	Fed	18	2.00	2.48	1.10	1	6	0.44	0.87
	Fasted	13	2.00	2.36	1.03	1	5.25		
2	Fed	10	3.00	3.10	0.99	2	5	0.81	0.77
	Fasted	9	2.00	2.77	0.52	2	6		
				Total sleep time (min)					
1	Fed	20	28.62	27.50	15.45	0	54	0.30	0.69
	Fasted	18	29.00	22.60	17.20	0	49		
2	Fed	10	65.50	61.00	23.33	4	93	0.70	0.42
	Fasted	10	58.50	53.50	29.06	0	91		
				Toe-pinch response in hindlimbs (min)					
1	Fed	20	6.50	7.60	8.17	0	24	not done	not done
	Fasted	18	0.00	4.42	7.04	0	20		
2	Fed	10	15.00	15.20	10.62	0	33	1.00	0.87
	Fasted	10	15.00	14.40	13.61	0	33		
				Toe-pinch response in forelimbs (min)					
1	Fed	18	8.25	10.10	9.28	0	27	0.37	0.96
	Fasted	18	10.00	9.30	7.96	0	22		
2	Fed	10	25.50	25.20	10.71	0	42	0.30	0.50
	Fasted	10	29.00	21.60	14.42	0	36		

Data were analyzed by using a linear regression model. For experiment 1, $n = 20$; K, 50 mg/kg; X 5 mg/kg. For experiment 2, $n = 10$; K, 70 mg/kg; X, 7 mg/kg.

Results

The prestudy feeding data indicated that rats ate, on average, 29.5% of their daily intake during the 3 h surrounding the change in light cycle from light to dark, validating the methodology behind the formation of the treatment groups and the reverse light cycle (Figure 1).

A large standard deviation existed for each variable regardless of whether the rats were in the fed or fasted groups. Rats that had a 0 for the time-to-recumbency variable were eliminated from the analysis, because these subjects did not become anesthetized. There was no significant difference between fed and fasted rats at either dose range for total sleep time or toe-pinch responses in the forelimbs in experiments 1 and 2 (Table 1). In experiment 1, toe-pinch responses in the hindlimbs were not analyzed statistically due to the high number of rats that did not lose the response (22 of 40). In addition, the study started with the intention to measure only toe-pinch responses in the hindlimbs, but because the first few rats consistently demonstrated a response to toe pinch of a hindlimb, the forelimb was added to determine whether toe-pinch response in the forelimbs differed between groups. In addition, descriptive statistics (mean, median, and standard deviation) are displayed in Table 1.

After the initial linear-regression model analysis, an exploratory statistical analysis was performed for each experiment. In this analysis, animals that showed 0 (did not respond at all to anesthesia) for total sleep time, toe pinch in forelimbs, and toe pinch in hindlimbs were excluded, and the linear regression repeated. The analysis did not reveal a statistically significant difference between fed and fasted rats. Descriptive statistics (mean, median, and standard deviation) are displayed in Table 2.

Because no previous data were available on which to base an a priori power analysis, we performed a posthoc analysis. Our power analysis revealed that a crossover study with an animal number of 10 had an 80% chance (that is, 0.80 power) of detecting a significant difference for time to recumbency if the true difference between groups was 2.06 min, for total sleep time if the true difference was 29.93 min, and for toe pinch (hindlimb or forelimb) if the true difference was 15.00 min. With a sample size of 15 animals, with the same power, the true differences in time to recumbency, total sleep time, and toe pinch (hindlimb or forelimb) would have to be 1.61, 23.37, and 11.68 min respectively. For a sample size of 20 and the same power, the true differences between time to recumbency, total sleep time, and toe pinch (hindlimb or forelimb) would have to be 1.37, 19.83, and 9.91 min, respectively.

Discussion

Variability in responses of individual rats to KX anesthesia is a documented phenomenon.[6, 10,11,19] We chose to examine the relationship of this variability to prandial status. Because rats

Table 2. Statistics comparing fed and fasted KX-anesthetized rats

Experiment		*n*	Median	Mean	1 SD	Minimum	Maximum
		Total sleep time (min)					
1	Fed	17	31.00	28.76	11.50	8	49
	Fasted	12	32.75	30.40	11.43	3.75	47
2	Fed	10	65.5	61.00	23.34	4	93
	Fasted	9	61.0	59.44	23.51	7	91
		Toe-pinch response in hindlimbs (min)					
1	Fed	10	11.5	11.6	5.08	3	21
	Fasted	6	13	13.33	5.16	6	20
2	Fed	8	18	19	7.91	9	33
	Fasted	6	26.5	24	7.54	13	33
		Toe-pinch response in forelimbs (min)					
1	Fed	12	14	13.58	7.29	3	24
	Fasted	11	14	14.27	4.68	6.5	21
2	Fed	9	26	28	6.40	22	42
	Fasted	8	30	27	10.04	4	36

Rats that did not become anesthetized were excluded from analysis. For experiment 1, $n = 20$; K, 50 mg/kg; X 5 mg/kg. For experiment 2, $n = 10$; K, 70 mg/kg; X, 7 mg/kg.

are not fasted routinely prior to anesthesia, the prandial status at the time of induction of anesthesia is usually unknown.

One potential reason for prandial status to affect anesthesia is that mesenteric blood flow increases after a meal in rats.[1] Because ketamine (the general-anesthetic component of KX) clearance is dependant partially on blood flow to the liver, it follows that prandial status could affect ketamine clearance and, therefore, response to anesthesia.[13] Previous studies show that propranolol, a drug that has a high hepatic extraction ratio (like ketamine), is cleared more quickly after a meal in people and in dogs.[3,15,16] If the same is true for ketamine and rats, we would expect that fed rats would have a longer time to recumbency and shorter total sleep time than would fasted rats, due to preferential blood flow to the gut after a meal and increased ketamine clearance in fed rats. In the current study, we did not use invasive techniques to measure pre- and postprandial hepatic blood flow or KX pharmacokinetics.

We used a reverse light cycle to maximize the amount fed rats ate in a short period of time. Measurement of stomach content or necropsy to show amount of food in stomachs was not used in the current study because of its crossover design. But preliminary measurements showed that rats ate about 30% of their total daily intake during the 3-h period including the 11th hour of the light phase and the first 2 h of the dark phase.

Potential interactions between ketamine and xylazine were controlled for in that each rat received the same dose of KX combined anesthesia during each of the 2 experiments. Xylazine may counter ketamine's peripheral vasopressor effects,[26] but there are no reports of xylazine's effects on ketamine's hepatic clearance or blood flow. Xylazine clearance in rats has not been well defined, but in an interspecific study of dog, cattle, sheep, and horse pharmakokinetics, xylazine had a large volume of distribution due to the drug's lipophilic properties, and drug elimination is attributed to a rapid metabolic clearance rather than excretion of unchanged xylazine or metabolites in the urine.[9]

Our ability to investigate only a single sex and stock for this initial study may somewhat limit the application of our findings to research in other stocks of rat. However, Sprague–Dawley rats represent a popular stock of rat used experimentally[22] and are used as a general multipurpose model with applications in virtually all disciplines of biomedical research. Male rats were used to avoid any cyclical reproductive variability among female rats. For these reasons, male Sprague–Dawley rats were an appropriate choice of rat for an initial investigation of our hypothesis. No previous studies were available to suggest that a fast of any length would make a difference in KX anesthesia. A short (3 h) fast was chosen because we expected it to cause minimal stress on rats, and the length of the fast represented a practical, easy change for investigators to make, had there been any significant findings to report. Whether a longer fast would have made a difference in anesthesia was beyond the scope of this study but is perhaps an interesting question to consider for future investigations.

A short period of fasting did not significantly reduce the variability in KX anesthesia at 2 different dosages, nor did it produce any significant difference in time to recumbency, total sleep time, or loss of toe pinch response (in either hindlimbs or forelimbs) when compared with fed rats. Consistent with previous reports, some rats in our study did not become recumbent or achieve surgical anesthesia at all.[6,11,19,23] The lack of response could not be attributed to any particular treatment group or individual animal.

One obvious reason for lack of response to an intraperitoneal injectable anesthetic is injection failure (injection into the muscle or subcutaneous tissue). The crossover design of this study prevented investigation of injection failures in the rats by direct visualization. Although the failure rate of intraperitoneal injections in rodent studies has been reported as low as 1% to 2% or as high as 20%,[8] it can be reduced to almost 0 when extreme care is used.[14,20] In the current study, a single trained investigator carefully administered all injections, in an attempt to reduce variability that could occur between administrators. Previous studies show that fasting a rat for as briefly as 4 h [similar to the length of fasting in this study (3 h)] can decrease injection error.[25] The results of the current study did not indicate a significant difference between experimental groups.

A posthoc power study confirmed that the difference in total sleep time between groups would need to be quite long (20 to 40

min) when 10 to 20 rats are used per group to detect a significant difference with a power of 0.8. Because the difference in total sleep time between the fed and fasted groups likely would be only a few minutes, it would not be clinically relevant or useful to repeat our study using a greater number of animals.

Our study did not find a significant difference between fed and fasted rats and therefore does not support use of a short fast for purposes of reducing KX anesthetic variability in male Sprague–Dawley rats.

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