

Acute Hyperglycemia Induced by Ketamine/Xylazine Anesthesia in Rats: Mechanisms and Implications for Preclinical Models

JOY K. SAHA,¹ JINQI XIA, JANET M. GRONDIN, STEVEN K. ENGLE, AND JOSEPH A. JAKUBOWSKI

BioTechnology Discovery Research, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285

The effects of anesthetic agents, commonly used in animal models, on blood glucose levels in fed and fasted rats were investigated. In fed Sprague-Dawley rats, ketamine (100 mg/kg)/xylazine (10 mg/kg) (KX) produced acute hyperglycemia (blood glucose 178.4 ± 8.0 mg/dl) within 20 min. The baseline blood glucose levels (104.8 ± 5.7 mg/dl) reached maximum levels (291.7 ± 23.8 mg/dl) at 120 min. Ketamine alone did not elevate glucose levels in fed rats. Isoflurane also produced acute hyperglycemia similar to KX. Administration of pentobarbital sodium did not produce hyperglycemia in fed rats. In contrast, none of these anesthetic agents produced hyperglycemia in fasted rats. The acute hyperglycemic effect of KX in fed rats was associated with decreased plasma levels of insulin, adrenocorticotrophic hormone (ACTH), and corticosterone and increased levels of glucagon and growth hormone (GH). The acute hyperglycemic response to KX was dose-dependently inhibited by the specific α_2 -adrenergic receptor antagonist yohimbine (1–4 mg/kg). KX-induced changes of glucoregulatory hormone levels such as insulin, GH, ACTH, and corticosterone were significantly altered by yohimbine, whereas the glucagon levels remained unaffected. In conclusion, the present study indicates that both KX and isoflurane produce acute hyperglycemia in fed rats. The effect of KX is mediated by modulation of the glucoregulatory hormones through stimulation of α_2 -adrenergic receptors. Pentobarbital sodium did not produce hyperglycemia in either fed or fasted rats. Based on these findings, it is suggested that caution needs to be taken when selecting anesthetic agents, and fed or fasted state of animals in studies of diabetic disease or other models where glucose and/or glucoregulatory hormone levels may influence outcome and thus interpretation. However, fed animals are of value when

exploring the hyperglycemic response to anesthetic agents. *Exp Biol Med* 230:777–784, 2005

Key words: acute hyperglycemia; hypoinsulinemia; ketamine/xylazine anesthesia; anesthetic agents; fed animals; fasted animals; isoflurane; pentobarbital sodium; glucoregulatory hormones

Commonly used anesthetics can differentially affect a number of physiological parameters in experimental models; these parameters include cardiovascular, neurohumoral, and behavioral. It is possible that the outcome of studies performed under certain anesthetics and using different therapeutic interventions may be influenced by different anesthetic approaches. Ketamine is a commonly used short-acting anesthetic and analgesic agent that induces a trance-like anesthetic state known as dissociative anesthesia in both animals and humans (1, 2). Xylazine is considered safe when used alone or in combination with other anesthetics and analgesics such as ketamine or isoflurane in animal research. Co-administration of ketamine with xylazine (KX, ketamine/xylazine anesthesia) is a commonly used anesthetic regimen in domestic and laboratory animals including mice and rats. The sedative and muscle-relaxing properties of xylazine are beneficial in reducing side effects of ketamine such as tremor and muscle rigidity (1, 3). Plasma glucose homeostasis is regulated by complex neurohumoral control mechanisms. Plasma glucose concentrations are maintained by the release of several glucose-raising hormones such as glucagon, catecholamines, adrenocorticotrophic hormone (ACTH), growth hormone (GH), and cortisol under certain conditions associated with stress (4). Administration of xylazine alone may cause hyperglycemia associated with a reduction in plasma insulin levels without any change in glucagon concentration in dogs and cattle (5, 6).

Despite the various reports describing the interference of the neuroendocrine system in the effect of KX in large

¹ To whom correspondence should be addressed at Eli Lilly and Company, BioTDR, DC 0444, Lilly Corporate Center, Indianapolis, IN 46285. E-mail: saha@lilly.com

Received May 25, 2005.
Accepted July 13, 2005.

1535-3702/05/23010-0777\$15.00
Copyright © 2005 by the Society for Experimental Biology and Medicine

animals (7–11), little is known in small animals such as rats and mice, which are widely used in preclinical research. Recent studies have revealed that KX may influence the outcome of several disease modifications by surgery or by drugs in laboratory small animals. KX has been reported to increase the infarct size in cerebral ischemia (12) and influences lipopolysaccharide-induced endotoxemia (13) as compared to other anesthesia. In our own experiments in rats and mice, commonly used laboratory anesthetic agents such as KX and isoflurane had dramatic effects on blood glucose levels of rats or mice depending on the fed or fasted state of the animals. Little is known regarding the mechanism of hyperglycemia induced by KX in rats. No attempts have been made so far to reveal the mechanism of KX or other commonly used anesthetic-induced hyperglycemia in rats.

Our initial observations led us to investigate (i) the effect of several anesthetic agents on blood glucose levels and (ii) the involvement of glucoregulatory hormones in both fed and fasted rats. The present study also utilized the α_2 -adrenoceptor antagonists to elucidate the mechanism of changes in blood glucose and glucoregulatory hormone levels induced by KX in rats.

A preliminary report of part of these results has been presented (Experimental Biology-2004 conference, Ref. 14).

Materials and Methods

General Animal Procedures. Experiments were carried out in male Sprague-Dawley rats (Charles River, Portage, MI) weighing 200–250 g. Following shipment all animals were acclimatized in the animal care facility for at least 1 week before any studies. The rats were maintained on a regular 12-hr dark/light cycle (6 PM to 6 AM) with access to food and water *ad libitum*, and the experiments were performed at approximately 10 AM each day. For the studies using fasted rats, food was withdrawn 18 hrs before the start of the experiment. The Institutional Animal Care and Use Committee (IACUC) of Eli Lilly and Company approved all protocols.

The animals were randomized according to their body weights and divided into several groups depending on the experimental design. Baseline blood glucose levels were measured immediately before the intramuscular (im) administration of ketamine (100 mg/kg)/xylazine (10 mg/kg) anesthesia (KX). The animals remained anesthetized throughout the entire duration of the study, typically 3 hrs from the onset of the administration of anesthesia. A maintenance dose (50% of the regular dose) of KX (im) was given 90 mins after induction. Rats received intraperitoneal (ip) administration of pentobarbital sodium at a dose of 60 mg/kg. A maintenance dose of 20 mg/kg ip was given 90 mins after the induction dose. For the studies with isoflurane, rats were placed in a plastic chamber specially designed for inhalation anesthesia. Isoflurane (2.5% in 1.5%

medical oxygen) was constantly delivered through a Vetamac Dual Flow Meter (MGX-VAD) Research Machine (Vetamac, Inc., Rossiville, IN) for the entire duration of the experiment (typically 3 hrs). In all experiments, body temperature was maintained at 36°–37°C by a thermostatically controlled warm water-circulating pad placed beneath the body. Yohimbine was administered subcutaneously (sc) 1 hr before the administration of KX at a dose volume of 1 ml/kg body wt. At the end of the experiment, blood samples were collected by cardiac puncture, and rats were euthanized by CO₂ and cervical dislocation.

Measurement of Blood Glucose. Blood glucose levels were measured by the Medisense Precision PCx (Abbott Medisense Division, Bedford, MA) blood glucose testing system (glucose strip method). The tip of the tail was snipped by sharp scissors and gently squeezed for a drop of blood. The strip was inserted in the slot of the hand-held machine, and the drop of blood was added to the strip. Within 20 secs, the device determined the blood glucose levels. The instrument's detection range is between 20 and 600 mg/dl (lower and upper detection limit of the instrument). Blood glucose levels were recorded in an Excel spreadsheet for subsequent statistical analysis. In some experiments, plasma glucose levels were also measured by a Hitachi 912 auto analyzer (Roche Diagnostics, Indianapolis, IN). The values obtained were similar with the values measured by the Medisense Precision PCx (data not shown).

Blood Collection and Handling. Blood samples were also collected by cardiac puncture at the end of the studies for a variety of biochemical analyses. Samples were collected in EDTA (0.5 M EDTA, pH 8.0, Gibco, Invitrogen Corporation, Grand Island, NY)-washed 10-ml syringes by cardiac puncture and transferred immediately into a 5-ml Monoject blood collection tube containing 7.5 mg EDTA (Sherwood Medical, St. Louis, MO). Blood samples were then centrifuged at 2500 g for 10 mins, and plasma was collected following standard protocols established in our laboratory.

Hormone Analysis. Plasma samples were analyzed by LINCOPLEX (Linco Research Inc., St. Charles, MO) for the determinations of the following hormones: GH, glucagon, insulin, ACTH, and corticosterone. All determinations were performed in a blinded fashion.

Drugs. The drugs used were ketamine hydrochloride injection (Fort Dodge Animal Health, Fort Dodge, IA), xylazine injection (The Butler Co., Columbus, OH), isoflurane (IsoFlu), pentobarbital sodium injection (Nembutal) (Abbott Laboratories, North Chicago, IL), and yohimbine hydrochloride (Sigma Chemical Co., St. Louis, MO). Ketamine and xylazine were mixed before simultaneous administration. Yohimbine was prepared in distilled water and vortexed to prepare a clear solution before use each day.

Data Analysis. Statistical analyses were performed by the Lilly Research Laboratories statistician. Statistical significance was determined based on analysis of variance

(ANOVA) from SAS (version 8.2) statistical analysis program (SAS Institute, Cary, NC). Box Cox transformation was applied to glucose, GH, glucagon, insulin, ACTH, and corticosterone because their data distributions were skewed. Data comparison was considered significant when the P value was <0.05 . Results were expressed as mean \pm SEM.

Results

Changes in Blood Glucose Levels Induced by Different Anesthetics. *Ketamine/Xylazine (KX)*.

Blood glucose levels were measured before and at different times over approximately 3 hrs following the administration of the anesthetic regimen. In the present study, elevation of blood glucose levels above 160 mg/dl (15) was considered to be hyperglycemic. Intramuscular administration of ketamine (100 mg/kg)/xylazine (10 mg/kg) anesthesia in fed rats increased the blood glucose levels in a time-dependent manner. The initial rise of blood glucose was observed after 20 min of KX administration. The peak level (291.7 ± 26.6 mg/dl) was reached at approximately 120 mins following the KX administration from its baseline levels of 104.8 ± 5.7 mg/dl. Blood glucose levels remained at an elevated level throughout the experiment (approximately 3 hrs) (Fig. 1A). Administration of the same dose of KX in fasted rats produced an increase in blood glucose levels within 20 mins, and the glucose level remained elevated until 150 mins. However, it never reached the hyperglycemic level (>160 mg/dl) throughout the study period (Fig. 1A).

Isoflurane. Similar to KX administration (im), inhalation of isoflurane (2.5% in 1.5% oxygen) resulted in a rapid onset of blood glucose elevation (Fig. 1B). The elevated levels of the blood glucose in fed rats lasted as long as the rats were kept under isoflurane anesthesia (Fig 1B). The blood glucose levels attained hyperglycemic level at 20 mins following anesthesia. The peak (232.4 ± 26.3 mg/dl) is reached at approximately 150 mins from its baseline levels of 125.0 ± 3.2 mg/dl. Blood glucose levels remained steady at the elevated level throughout the experiment. In contrast, the blood glucose levels of the fasted rats did not reach hyperglycemic levels at any time during the 3-hr study period. However, a nonsignificant increase in blood glucose levels was observed. It is evident from the comparison of Figure 1A and Figure 1B that the extent of increase in blood glucose levels in fed rats was less to some extent with isoflurane than with KX.

Pentobarbital Sodium. Pentobarbital sodium at its commonly used anesthetic dose was ineffective in producing hyperglycemia in either fed or fasted rats. As illustrated in Figure 1C, in fed rats, pentobarbital sodium (60 mg/kg, ip) produced an immediate but variable increase (non-significant) in blood glucose levels, which subsequently dropped down below the baseline levels. This fall in blood glucose levels occurred after approximately 90 mins of pentobarbital sodium and lasted throughout the whole duration (180 mins) of the experiment. In fasted rats, the

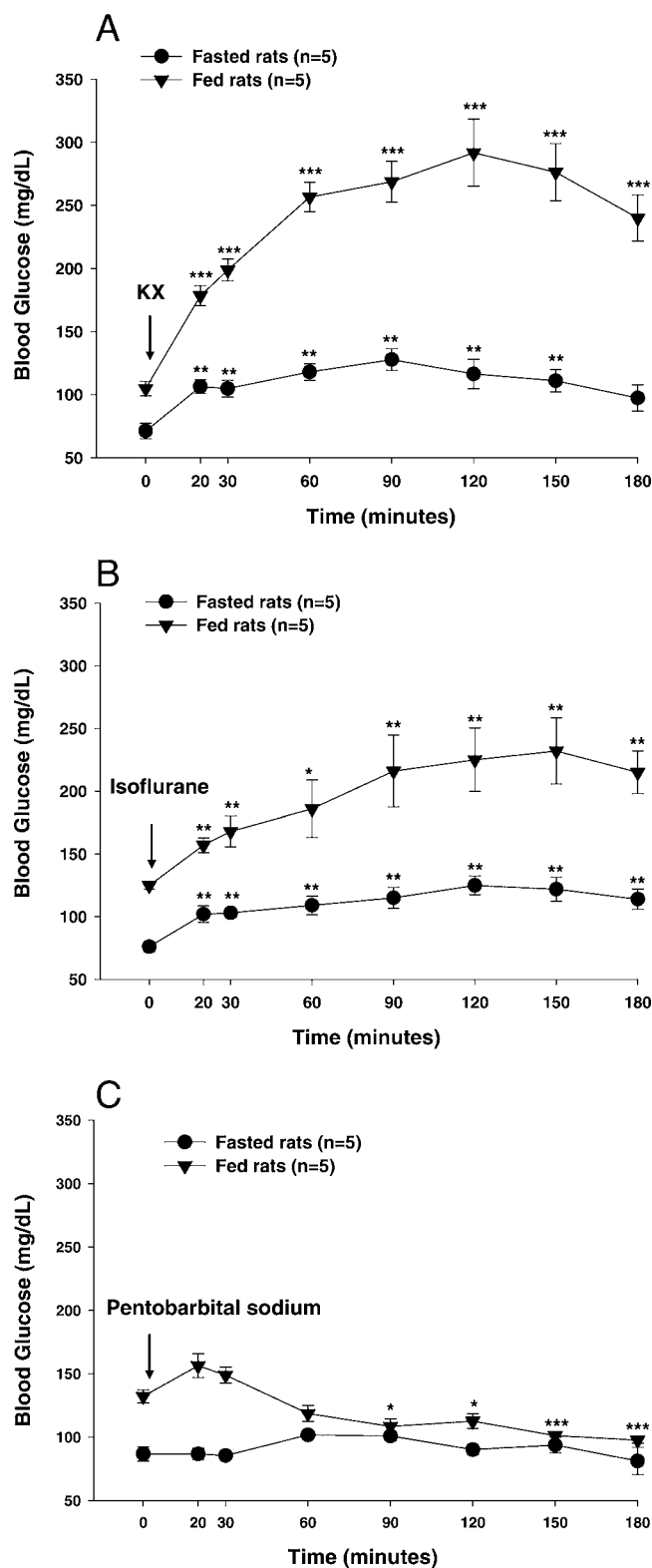


Figure 1. Time-dependent effect of (A) KX (100 mg/10 mg/kg, im), (B) isoflurane (2.5% in 1.5% oxygen inhalation), and (C) pentobarbital sodium (60 mg/kg, ip) on blood glucose levels in fasted (closed circle) and fed (closed triangle) rats. Each point represents mean \pm SEM of 5 animals for each treatment. Zero (0) time indicates baseline values. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs. control ($t = 0$) levels.

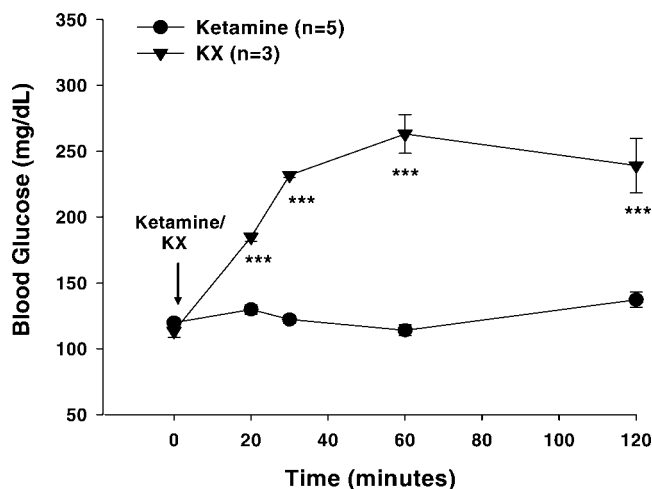


Figure 2. Effect of ketamine (100 mg/kg, im) alone and KX (100 mg/10 mg/kg, im) mixture on blood glucose levels in fed rats. Ketamine alone did not produce any change in the blood glucose levels, whereas KX produced hyperglycemia beginning at 20 min that persisted throughout the experimental period. Results are mean \pm SEM, $n = 3-5$ animals. Zero (0) time indicates baseline values. *** $P < 0.001$ vs. baseline ($t = 0$) and ketamine alone.

blood glucose levels remained unchanged at all time points following the administration of pentobarbital sodium.

Ketamine Alone Versus KX. Studies were extended to compare the effect of ketamine alone and KX as a combination in fed rats. Administration of ketamine alone did not produce any changes in blood glucose levels (Fig. 2), whereas KX produced significant increases in blood glucose levels in fed rats, confirming the previous data (Fig. 1A).

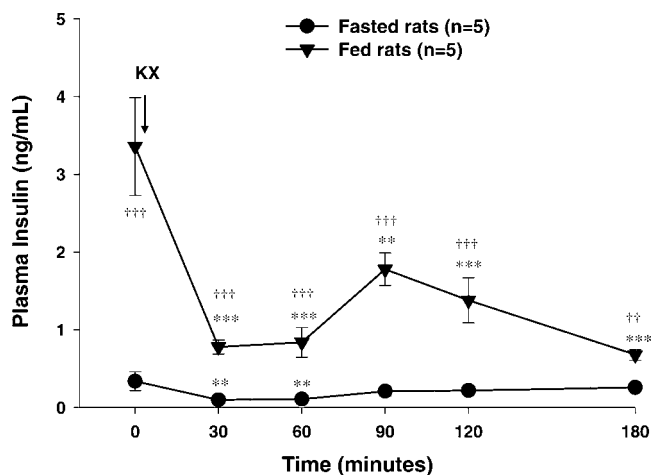


Figure 3. Time-dependent effect of KX on plasma insulin levels in fed (closed triangle) and fasted (closed circle) rats. Plasma insulin levels were significantly decreased following KX administration in fed rats and then rose substantially, although they remained significantly lower than the baseline levels ($t = 0$). Insulin levels changed significantly at earlier time after KX in fasted rats. Results are mean \pm SEM, $n = 5$ animals. Zero (0) time indicates baseline values. ** $P < 0.01$ and *** $P < 0.001$ vs. baseline ($t = 0$); ††† $P < 0.01$ and †††† $P < 0.001$ fed vs. fasted rats.

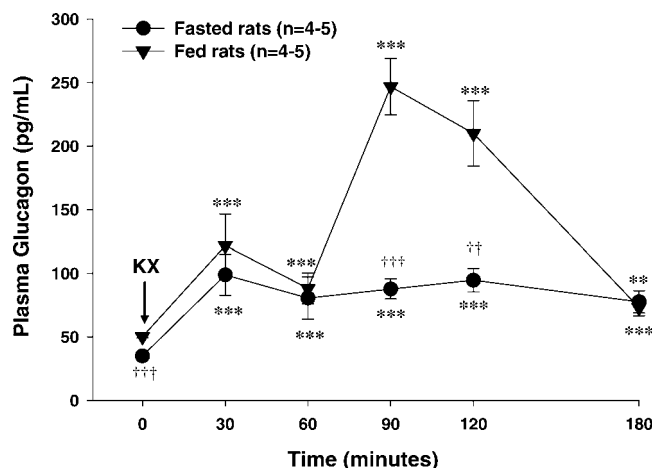


Figure 4. Time-dependent effect of KX on plasma glucagon levels in fed (closed triangle) and fasted (closed circle) rats. Plasma glucagon levels increased significantly from baseline at all time points after the initiation of anesthesia in both fed and fasted rats but was most notable between 60 and 180 mins in fed rats. Results are mean \pm SEM, $n = 4-5$ animals. Zero (0) time indicates baseline values. ** $P < 0.01$ and *** $P < 0.001$ vs. baseline ($t = 0$); ††† $P < 0.01$ and †††† $P < 0.001$ fed vs. fasted rats.

Effect of Ketamine/Xylazine on Plasma Hormone Levels.

To explore possible mechanisms of action of KX-induced hyperglycemia, the plasma levels of several hormones were determined in both fed and fasted rats. As shown in Figure 3, in fed rats, plasma insulin levels dropped dramatically (3.4 ± 0.6 to 0.8 ± 0.1 ng/ml, $P < 0.001$) within 30 mins of KX administration. Insulin levels recovered to some extent by 90 mins, showing a biphasic pattern, but remained significantly below the baseline levels. The baseline levels of insulin in fasted rats were substantially lower than those in the fed rats (0.3 ± 0.1 vs. 3.4 ± 0.6 , $P < 0.01$) and decreased initially (30–60 mins) but remained unchanged at all other times after KX (Fig. 3). KX administration in fed rats resulted in a variable but significant increase in plasma glucagon levels (Fig. 4). The highest increase occurred between 60 and 180 mins of the KX administration. The peak level (246.8 ± 22.2 pg/ml) was reached at 90 mins (Fig. 4). Even though the glucagon levels increased following KX administration in fasted rats, they were substantially lower than the levels observed in fed rats (Fig. 4). Overall GH levels increased significantly after KX administration in fed rats (Table 1). In fasted rats, the baseline GH levels were higher than those in fed rats and did decrease significantly at later time point following KX administration (Table 1). KX produced significant inhibition of ACTH levels at a later time point (180 mins) in fed rats. But KX produced a biphasic effect on ACTH levels in fasted rats, initially decreased and later on increased (Table 1). KX, however, produced significant reduction in corticosterone levels in both fed and fasted rats (Table 1).

Effect of α_2 -Adrenoceptor Antagonist Yohimbine on the Response to KX. Studies were performed with the selective α_2 -adrenoreceptor antagonist yohimbine.

Table 1. Changes in Hormone Levels Following Ketamine/Xylazine Anesthesia in Fed and Fasted Rats^a

Hormone	Time (min) after ketamine/xylazine anesthesia					
	Baseline (0)	30	60	90	120	180
GH (ng/dl)						
Fed	23.2 ± 3.9	70.3 ± 20.1 ***	81.8 ± 27.8 ***	37.0 ± 3.7 *	34.2 ± 2.4 *	50.1 ± 19.3 *
Fasted	58.2 ± 19.8	34.2 ± 3.5	31.7 ± 4.3	30.4 ± 3.4	36.5 ± 3.95	28.7 ± 5.2 *
ACTH (pg/ml)						
Fed	294.0 ± 28.3	213.8 ± 31.2	212.0 ± 29.7	245.2 ± 12.2	326.6 ± 29.1	181.7 ± 25.6 *
Fasted	176.0 ± 35.2	128.6 ± 18.6	161.2 ± 33.4 *	211.7 ± 16.7	240.4 ± 27.4	222.4 ± 44.1 *
Corticosterone (ng/ml)						
Fed	274.2 ± 22.8	239.8 ± 19.7	231.8 ± 19.6	194.0 ± 23.2	177.4 ± 18.2 *	133.5 ± 24.6 **
Fasted	284.0 ± 56.4	129.2 ± 52.4 **	157.6 ± 46.5	246.5 ± 10.4	271.2 ± 14.4	188.4 ± 41.9 *

^a Results are mean ± SEM ($n = 4-5$).

* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs. baseline ($t = 0$).

Fed rats were pretreated with different doses of yohimbine (1–4 mg/kg) or vehicle 1 hr before administration of KX. Acutely, yohimbine did not significantly alter baseline (0 min) blood glucose levels (Fig. 5). However, yohimbine pretreatment resulted in a significant inhibition of the hyperglycemic effect of KX in a dose-dependent manner. The inhibition was evident as early as 30 mins after KX administration at the high dose of yohimbine (2–4 mg/kg), but the inhibitory effect was delayed with a lower dose of yohimbine (1 mg/kg). Additional studies were performed to determine the effect of yohimbine on KX-induced changes in glucoregulatory hormone levels. Rats anesthetized with KX were pretreated (1 hr) with different doses of yohimbine, and blood samples were collected 180 mins after KX to determine the level of hormones. Yohimbine pretreatment produced a dose-dependent increase in insulin levels. Higher dose resulted in a significant inhibition of GH

levels. Glucagon levels remained unchanged at all doses of yohimbine. However, yohimbine produced significant inhibition in ACTH levels at the lower doses. The corticosterone levels were also inhibited at all three doses of yohimbine. (Table 2).

Blood Glucose and Plasma Hormone Levels in Fed and Fasted Rats. The preceding results showed that the baseline (0 min) blood glucose levels were significantly higher in fed rats compared to fasted rats (120.7 ± 4.0 vs. 78.1 ± 3.5 mg/dl, $n = 15$, $P < 0.001$). In separate experiments, the baseline blood glucose and hormone levels in fed and fasted rats were determined within 5 mins of pentobarbital sodium anesthesia. Pentobarbital sodium did not produce any changes in blood glucose levels in either fed or fasted rats (Fig. 1C). We found that only insulin (3.4 ± 0.6 vs. 0.3 ± 0.1 ng/ml, $P < 0.001$), glucagon (50.2 ± 1.1 vs. 35.0 ± 1.6 pg/ml, $P < 0.001$), and ACTH (294.0 ± 28.3 vs. 176.0 ± 35.2 pg/ml, $P < 0.05$) levels in fed and fasted rats, respectively, were significantly higher in fed compared to fasted rats. GH (23.2 ± 3.9 vs. 58.2 ± 19.8 , $P < 0.01$) levels were significantly lower in fed rats compared to fasted rats. Corticosterone levels were not significantly different between the fed and fasted rats.

Discussion

In preclinical animal models, it is generally assumed that physiological parameters of the animal under general anesthesia represent the basal state of the animal (before institution of the disease model). However, different anesthetics can variably affect cardiovascular, neurohumoral, and behavioral parameters. Similarly, if the fed and fasted states of animals are chosen arbitrarily, it may cause a number of changes in physiological parameters. Therefore, it is probable that the type of anesthetic agents and/or fed and fasted states of animals used in the studies with different therapeutic interventions may influence the outcome of the observations. Our studies were prompted by the observation that commonly used anesthetic agents such as KX and isoflurane had profound effects on blood glucose levels in

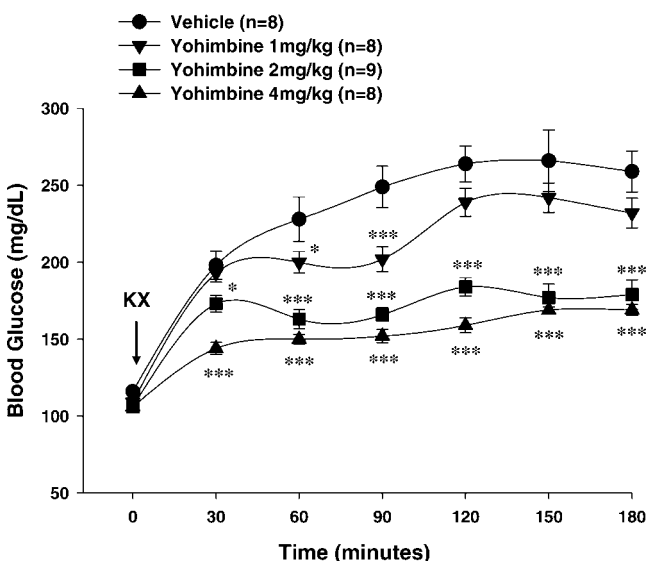


Figure 5. Dose-dependent inhibitory effect of yohimbine pretreatment (1–4 mg/kg) on the hyperglycemic response to KX in fed rats. Results are mean ± SEM, $n = 8-9$ animals. * $P < 0.05$ and *** $P < 0.001$ vs. vehicle.

Table 2. Dose-Dependent Effect of Yohimbine on Gluco-Regulatory Hormone Levels at 180 Min Following Administration of Ketamine/Xylazine Anesthesia in Fed Rats^a

Hormone	Dose of yohimbine			
	Vehicle	1 mg/kg	2 mg/kg	4 mg/kg
GH (ng/dl)	26.7 ± 2.8	25.4 ± 2.8	19.7 ± 3.0	3.9 ± 2.8 ***
Insulin (ng/ml)	0.6 ± 0.2	1.2 ± 0.2 *	1.2 ± 0.2 *	2.0 ± 0.2 ***
Glucagon (pg/ml)	71.6 ± 6.2	77.0 ± 6.2	61.5 ± 7.1	81.6 ± 6.2
ACTH (pg/ml)	354.2 ± 31.5	177.4 ± 31.5***	181.6 ± 36.3 ***	336.1 ± 31.5
Corticosterone (ng/ml)	237.5 ± 10.7	175.5 ± 10.7 ***	159.6 ± 11.5 ***	203.0 ± 10.7*

^a Results are mean ± SEM ($n = 6-8$).

* $P < 0.05$ and *** $P < 0.001$ vs. vehicle.

fed rodents (14) and were aimed to (i) investigate and compare the effects of different anesthetics such as KX, isoflurane, and pentobarbital sodium in a commonly used laboratory animal, the rat, (ii) investigate the influence of both fed and fasted conditions on determining the effects of different anesthetics, and (iii) investigate the mechanism of anesthesia-induced acute hyperglycemia in rats. The present investigation demonstrates that administration of KX in fed rats produced significant increases in blood glucose levels leading to acute hyperglycemia (blood glucose greater than 160 mg/dl), which was found to develop in a time-dependent manner. In contrast, when administered to fasted rats KX did not produce acute hyperglycemia, although small increases in blood glucose levels were noted. Inhalation of the volatile anesthetic isoflurane in fed rats also produced acute hyperglycemia similar to KX, which was also attenuated in fasted rats. In contrast, pentobarbital sodium had little effect, producing an initial small non-significant increase in blood glucose followed by a delayed decrease in blood glucose levels, whereas in fasted rats, there was no effect.

Xylazine, an analogue of clonidine, is a known α_2 -adrenergic agonist. Xylazine induces hyperglycemia and is associated with a decrease in plasma insulin in dogs (11) and cats (16). To date, all studies reported were performed in veterinary animals, and little is known about the effect of xylazine alone or in combination with ketamine in the rat, a commonly used laboratory animal. The present study clearly demonstrates that KX (at doses typically used for induction of anesthesia) produces profound hyperglycemia in fed rats. This was associated with decreases in plasma insulin, ACTH, and corticosterone and an increase in plasma glucagon and GH levels. KX-induced hyperglycemia in fed rats may, therefore, result from the increased glucose output secondary to increased hepatic glucose production (glycogenolysis and gluconeogenesis) and/or suppression of peripheral glucose uptake secondary to decreased insulin levels. On the other hand, in fasted rats KX also produced significant changes in all the gluco-regulatory hormone levels along with a small increase in blood glucose levels at all time points. Because fasted rats have reduced glycogen

stores (17), the contribution of glycogenolysis to increase blood glucose levels in fasted rats is limited.

We observed that KX-induced hyperglycemia was dose-dependently inhibited by the specific α_2 -adrenergic antagonist yohimbine concomitant with a significant inhibition of GH, ACTH, and corticosterone levels and rise in insulin level. Therefore, it may be inferred that the KX-induced changes in hormones levels are in large part mediated by the α_2 -adrenoceptors in the fed rats. Xylazine has been reported to increase the release of GH in cattle and dogs (9, 18). Later studies suggested that xylazine stimulates GH release via the α_2 -adrenoceptor pathway in cattle, but the mechanism of hyperglycemia induced by xylazine still remains to be fully elucidated (9). The present study strongly suggests the involvement of insulin, GH, ACTH, and corticosterone released via the α_2 -adrenoceptor pathway in producing KX-induced hyperglycemia as suggested by previous studies in cattle and dogs (9, 18).

Activation of α_2 -adrenoceptor has been reported to cause either an increase, a decrease, or no change in glucagon levels. Increased glucagon levels in association with decreased insulin levels may account for the hyperglycemic effect of α_2 -adrenoceptor agonist (19). Clonidine, an α_2 -adrenoceptor agonist, increased glucagon levels and was inhibited by yohimbine, suggesting the involvement of α_2 -adrenoceptor in mediating the effects of clonidine (20). There are conflicting reports regarding the involvement of glucagon in mediating the hyperglycemic effect of xylazine, the α_2 -adrenoceptor agonist (6, 21). It has been reported that xylazine did not change glucagon levels and remained unaffected by yohimbine in dogs (11, 22). In the present study, although KX produced a significant increase in glucagon levels (and also a decrease in insulin level), it remained unaffected by yohimbine. It is probable that glucagon levels are not regulated by the α_2 -adrenergic receptor as suggested by the findings of Ambrisko and Hikasa (11) in dogs. Further studies are needed to clarify the role of glucagon in KX-induced hyperglycemia in fed rats. Ketamine is a dissociative anesthetic agent and is used alone as a brief sedative for nonsurgical procedures. It is also used routinely in combination with xylazine (13). Our data indicate that it probably does not contribute directly to the

changes in blood glucose levels associated with KX, and the data are consistent with previous results in cats and baboons (23, 24).

Volatile anesthetics such as isoflurane and halothane are widely used as anesthetic agents in studies involving rodents and other laboratory animals. Previous studies have indicated that the volatile anesthetics halothane, in fed rats (25), and isoflurane, in fed dogs (26), produce a small increase in blood glucose levels. There are no studies that described the effect of isoflurane in laboratory animals such as rats and mice. The present study is the first to demonstrate that isoflurane, at a commonly used dose, produces sustained increases (>200 mg/dl) in blood glucose levels for up to 3 hrs in fed rats in contrast to a lesser degree (<130 mg/dl) in fasted rats. This response pattern is similar to that observed under KX; however, the present study has not explored the mechanistic basis for isoflurane-induced hyperglycemia.

In the present studies, administration of pentobarbital sodium in fasted rats produced no change in the steady-state blood glucose levels, which supports earlier observations (14, 27–29). In fed rats, administration of pentobarbital sodium produced an initial small increase in blood glucose level that was followed by a subsequent decrease. This secondary drop in blood glucose level observed in the present study differs from what was seen in the earlier studies (28) and may result from the effect of the supplemental administration of pentobarbital sodium during the course of the study. Previous studies showed that injection of pentobarbital sodium in fed rats produced transient increases in blood glucose level (within 5 mins), which then returned to a steady-state level over an hour (30, 31), dissimilar to the present study. Similar to the present findings, pentobarbital sodium in fasted rats maintained baseline blood glucose levels for a period of 5 hrs (27).

Acute hyperglycemia may influence the outcome in a number of critical illnesses. Acute hyperglycemia is a predictor of mortality after acute myocardial infarction (32, 33), stroke (34, 35), and following cardiac surgery in critically ill patients (36). The adverse effect of hyperglycemia is also reflected in animal models of myocardial infarction (37, 38). Therefore, great care must be taken in these models to control acute glucose levels by avoiding the use of agents such as KX or isoflurane in fed rats because the associated acute hyperglycemia will influence the outcome and will confound interpretation of the data.

The present study compared the baseline blood glucose levels in both fed and fasted states of the rats. As expected, it was observed that the fed rats had higher blood glucose levels than the fasted rats. Further studies examining the baseline levels of a number of glucoregulatory hormones revealed that insulin, glucagon, and ACTH were significantly higher at the baseline (0 min) levels in fed rats as compared to fasted rats. The present study further investigated the association of corticosterone and GH levels with altered blood glucose levels. GH levels were significantly

lower in fed rats compared to fasted animals, whereas corticosterone levels remained unchanged. However, to avoid substantial variations in the outcome resulting from the influence of several factors such as anesthesia-induced hyperglycemia and changes in glucoregulatory hormones, precaution should be taken in using the fed and fasted state of the experimental animals.

In conclusion, the present studies demonstrate that commonly used anesthetic agents, namely KX and isoflurane, produce acute hyperglycemia in fed rats, whereas pentobarbital sodium did not. In contrast, none of the anesthetic agents have produced any acute hyperglycemia in fasted rats. The acute hyperglycemic effect of KX in part reflects α_2 -adrenoceptor-dependent changes of glucoregulatory hormones such as insulin, corticosterone, GH, and ACTH. Because acute hyperglycemia can influence the outcome in a variety of animal models, care must be taken in selecting the anesthetic agents as well as the fed or fasted state of the experimental animals. Based on the present observations, it is recommended that to avoid the secondary hyperglycemic effects of anesthetic agents, experiments should be performed using KX, isoflurane, or pentobarbital sodium in case of fasted rats. If fed rats are used, pentobarbital sodium should be used. Moreover, use of fed rats will be beneficial to determine hyperglycemic effect of any anesthetic agents.

We thank Dr. Yunfei Chen and Mr. Jason R. Manro for their help in statistical analysis of the data.

1. Wright M. Pharmacologic effects of ketamine and its use in veterinary medicine. *J Am Vet Med Assoc* 180:1462–1471, 1982.
2. Bergman SA. Ketamine: review of its pharmacology and its use in pediatric anesthesia. *Anesth Prog* 46:10–20, 1999.
3. Lei H., Grinberg O, Nwaigwe CI, Hou Hg, Williams H, Swartz HM. The effects of ketamine-xylazine anesthesia on cerebral blood flow and oxygenation observed using nuclear magnetic resonance perfusion imaging and electron paramagnetic resonance oximetry. *Brain Res* 913:174–179, 2001.
4. Toso CF, Rodriguez RR, Renaud AR, Marquez AG, Linares LM. Adrenocorticotrophic hormone, cortisol and catecholamine concentrations during insulin hypoglycemia in dogs anaesthetized with thiopentone. *Can J Anaesth* 40:1084–1091, 1993.
5. Symonds HW, Mallinson CB. The effect of xylazine and xylazine followed by insulin on blood glucose and insulin in the dairy cow. *Vet Rec* 102:27–29, 1978.
6. Goldfine ID, Arieff AI. Rapid inhibition of basal and glucose stimulated insulin release by xylazine. *Endocrinology* 105:920–920, 1979.
7. Hsu WH, Hummel SK. Xylazine-induced hyperglycemia in cattle: a possible involvement of α -adrenergic receptors regulating insulin release. *Endocrinology* 109:825–829, 1981.
8. Muggaberg J, Brockman RP. Effect of adrenergic drugs on glucose and plasma glucagon and insulin response to xylazine in sheep. *Res Vet Sci* 33:118–120, 1982.
9. Kasuya E, Hodate K, Matsumoto M, Sakaguchi M, Hashizume T, Kanematsu S. The effects of xylazine on plasma concentrations of growth hormone, insulin-like growth factor-1, glucose and insulin in calves. *Endocr J* 43:145–149, 1996.

10. Kasuya E, Hodate K, Matsumoto M, Sakaguchi M, Hashizume T, Kanematsu S. Effects of atipamezole, an α -adrenergic antagonist, and somatostatin on xylazine-induced growth hormone release in calves. *Endocr J* 43:551–556, 1996.
11. Ambrisko TD, Hikasa Y. Neurohumoral and metabolic effects of medetomidine compared with xylazine in beagle dogs. *Can J Vet Res* 66:42–49, 2002.
12. Kawai N, Keep RF, Betz AL. Hyperglycemia and the vascular effects of cerebral ischemia. *Stroke* 28:149–154, 1997.
13. Helmer KS, Cui Y, Chang L, Dewan A, Mercer DW. Effect of ketamine/xylazine on expression of tumor necrosis factor- α inducible nitric oxide synthase, and cyclo-oxygenase-2 in rat gastric mucosa. *Shock* 20:63–69, 2003.
14. Saha JK, Xia J, Grondin J, Engle SK, Jakubowski JA. Anesthesia-induced acute hyperglycemia in rodent. *FASEB J* 18:A578, 2004.
15. Nauck MA, Walberg J, Vethacke A, El-Quaghli A, Senkal M, Holst JJ, Galwitz JB, Schmidt WT, Schmiegel W. Blood glucose control in healthy subject and patients receiving intravenous glucose infusion or total parenteral nutrition using glucagon-like-peptide-1. *Regul Peptides* 118:89–97, 2004.
16. Feldberg W, Symonds HW. Hyperglycemic effect of xylazine. *J Vet Pharmacol Ther* 3:197–202, 1980.
17. Geary N, Langhans W, Sharer E. Metabolic concomitants of glucagon-induced suppression of feeding in the rat. *Am J Physiol* 241:R330–R335, 1981.
18. Hampshire J, Altszuler N. Clonidine or xylazine as provocative test for growth hormone secretion in the dog. *Am J Vet Res* 42:1073–1076, 1981.
19. Velliquette RA, Ernsberger P. The role of I_1 -imidazoline and α -2 adrenergic receptors in the modulation of glucose metabolism in the spontaneously hypertensive obese rat model of metabolic syndrome X. *J Pharmacol Exp Ther* 306:646–657, 2003.
20. Saito M, Saitoh T, Inoue S. Alpha-2 adrenergic modulation of pancreatic glucagon secretion in rats. *Physiol Behav* 51:1165–1171, 1992.
21. Brockman RP. Effect of xylazine on plasma glucose, glucagon, and insulin concentrations in sheep. *Res Vet Sci* 30:383–384, 1981.
22. Ambrisko TD, Hikasa Y. The antagonistic effects of atipamezole and yohimbine on stress-related neurohormonal and metabolic responses induced by medetomidine in dogs. *Can J Vet Res* 67:64–67, 2002.
23. Hsu WH, Hembrough FB. Intravenous glucose tolerance test in cats: influenced by acetylpromazine, ketamine, morphine, thiopental, and xylazine. *Am J Vet Res* 43:2060–2061, 1982.
24. Lehmann R, Wagner JL, Fernandez LA, Bourgoignie JJ, Rocordi C, Alejandro R, Kenyon NS. Effects of ketamine sedation on glucose clearance, insulin secretion and counter regulatory hormone production in baboons (*Papio hamadryas*). *J Med Primatol* 26:312–321, 1997.
25. Heath DF, Frayn KN, Rose JG. Glucose turnover in the post-absorptive rat and the effects of halothane anesthesia. *Biochem J* 162:653–657, 1977.
26. Horber FF, Krayner S, Rehder K, Haymond MW. Anesthesia with halothane and nitrous oxide alters protein and amino acid metabolism in dogs. *Anesthesiology* 69:319–326, 1988.
27. Bailey CJ, Atkins TW, Matty AJ. Blood glucose and plasma insulin levels during prolonged pentobarbitone anesthesia in the rat. *Endocrinol Exp* 9:177–185, 1975.
28. Johansen O, Vaaler S, Jorde R, Reikeras O. Increased plasma levels after Hypnorm anesthesia, but not after pentobarbital anesthesia in rats. *Lab Anim* 28:244–248, 1994.
29. Illera JC, Gil AG, Illera M. The effects of different anesthetic treatments on the adreno-cortical functions and glucose levels in NZW rabbits. *J Physiol Biochem* 56:329–336, 2000.
30. Penicaud L, Ferre P, Kande J, Leturque A, Issad T, Girard J. Effect of anesthesia on glucose production and utilization in rats. *Am J Physiol* 252:E365–E369, 1987.
31. Clark PW, Jenkins AB, Kraegen EW. Pentobarbital reduces basal liver glucose output and its insulin suppression in rats. *Am J Physiol* 258:E701–E707, 1990.
32. Capes SE, Hunt D, Malmberg K, Gerstein HC. Stress hyperglycemia and increased risk after myocardial infarction in patients without diabetes: a systematic overview. *Lancet* 355:773–778, 2000.
33. Davies MJ, Lawrence IG. DIGAMI (diabetes mellitus, insulin glucose infusion in acute myocardial infarction): theory and practice. *Diabetes Obes Metab* 4:289–295, 2001.
34. Scott JF, Robinson GM, French JM, O'Connell JE, Alberti KGMM, Gray CS. Blood pressure response to glucose potassium insulin therapy in patients with acute stroke with mild to moderate hyperglycemia. *J Neurol Neurosurg Psychiatry* 70:401–404, 2001.
35. Bruno A, Williams LS, Kent TA. How important is hyperglycemia during acute brain infarction? *Neurologist* 10:195–200, 2004.
36. Van Den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande C, Lauwers P, Bouillon R. Intensive insulin therapy in critically ill patients. *N Engl J Med* 345:1359–1367, 2001.
37. Marfella R, D'amico M, Di Filippo C, Piegari E, Nappo F, Esposito K, Berrino L, Rossi F, Giugliano D. Myocardial infarction in diabetic rats: role of hyperglycemia on infarct size and early expression of hypoxia-inducible factor. *Diabetologia* 45:1172–1181, 2002.
38. Shiomi T, Tsutsui H, Ikeuchi M, Matsusaka H, Hayashidani S, Suematsu N, Wen J, Kubota T. Streptozotocin-induced hyperglycemia exacerbates left ventricular remodeling and failure after experimental myocardial infarction. *J Am Coll Cardiol* 42:165–172, 2003.