A Model of Controlled Acute Hyperglycemia in Rats: Effects of Insulin and Glucagon-Like Peptide-1 Analog

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ABSTRACT
A rodent model of controlled acute hyperglycemia that is sensitive to glucose-lowering agents insulin and glucagon-like peptide-1 (GLP-1) analog has been developed. The studies show that anesthesia could be induced in fasted rats with ketamine (100 mg/kg) plus a low dose of xylazine (5 mg/kg) without inducing the acute hyperglycemia typically associated with these agents. Under these conditions, continuous infusion of glucose (10 and 20%) via the jugular vein for 30 to 150 min induced hyperglycemia in a time-dependent fashion. Administration of "loading" boluses of glucose (0.2–0.6 ml of a 20% solution) prior to continuous infusion of 10% glucose produced more immediate and sustained hyperglycemia. Plasma levels of a variety of glucoregulatory and stress hormones such as insulin, growth hormone, glucagon, and corticosterone were determined. Only glucagon levels changed significantly during induction and maintenance of hyperglycemia. The infusion of insulin (0.1 U/kg/h) or GLP-1 analog (10 μg/kg/h) effectively lowered blood glucose from its elevated levels. Insulin produced a significant increase in glucagon levels, and GLP-1 analog produced a significant increase in insulin levels without any change in other glucoregulatory and stress hormone levels. In conclusion, the present studies identified a novel approach for the induction of anesthesia and surgical manipulations without inducing hyperglycemia and further defined an approach for producing acute hyperglycemia in a controlled fashion in rodents. This model will be beneficial to study the influence of hyperglycemia in acute models of critical illness where hyperglycemia develops following the precipitating event. This model was responsive to insulin and GLP-1 analog, both of which were effective in ameliorating hyperglycemia.

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 in humans (Wright, 1982; Bergman, 1999). Ketamine also induces side effects such as tremor, muscle rigidity, and excitement during recovery that may cause an increase in muscle tone (Wright, 1982). Xylazine is a commonly used sedative, muscle relaxant, and analgesic agent that is used for gastrointestinal surgery and endoscopy due to its gastric and intestinal motility inhibitory properties (Greene, 1999). Coadministration of ketamine with xylazine [ketamine/xylazine anesthesia (KX)] is commonly used in laboratory animals such as mice and rats to induce anesthesia and to enable surgical interventions to induce disease and develop animal models of human diseases. It has been reported that administration of xylazine alone or in combination with ketamine may cause hyperglycemia in dogs and cattle (Symonds and Mallinson, 1978; Goldfine and Arieff, 1979). Recently, we have demonstrated that KX produces acute hyperglycemia in fed rats and may be used as an animal model of acute hyperglycemia to test the efficacy and mechanism of glucoregulatory and other therapeutic agents (Saha et al., 2005b).

Surgery, like trauma, may cause marked changes in metabolism of which accelerated glucose synthesis is an important change after surgery and occurs at the expense of body protein and energy stores (Black et al., 1982; Brandi et al., 1993). These changes may cause alterations in glucoregulatory stress hormones and cytokines that may lead to greater morbidity and slower recovery from disease (Chernow et al., 1987; Thorell et al., 1993). Bruno et al. (2002) have reported that only 21% of stroke patients presenting with hyperglycemia had a history of diabetes. Likewise, Van den Berghe et al. (2001) reported that only 13% of critically ill patients with hyperglycemia were diabetic. It has been reported that hyperglycemia develops in response to the trauma of the acute illness (Hirsch, 2002), and such hyperglycemia has been reported to be associated with poor clinical outcome in post surgical patients (Van den Berghe et al., 2001) and in patients following acute myocardial infarction (Capes et al.,...
mixed together and injected i.m. Blood glucose levels were measured just prior to and at several times after KX administration. The rats remained unconscious for the entire duration (3 h) of the experiments. The rats typically received a maintenance dose of KX once within the 3-h period. Exogenous glucose or vehicle infusion was administered through jugular vein catheters, and the effect on blood glucose levels was determined. In certain experiments, the ability of insulin or GLP-1 analog to impact hyperglycemia was tested. At the end of the experiments, blood samples were collected for measurement of a variety of hormones, and the rats were euthanized by CO2 followed by 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 snapped with sharp scissors and gently squeezed for a drop of blood. The strip was inserted into the machine, and the drop of blood was placed on the strip. Within 20 s, the instrument measured and displayed the blood glucose level. The variability of the blood glucose levels measured with this instrument is within the range of 2 to 5% of the average value. Blood glucose levels were recorded in an Excel spreadsheet for further analysis.

Catheterization of the Jugular Vein. Following induction of anesthesia as described above, the skin and the underlying muscle at both sides of the neck region were dissected. Under a dissecting microscope, we identified and isolated the external jugular veins on each side. A polyethylene catheter (PE-20; BD Biosciences, San Jose, CA) containing heparin (100 U/ml)–saline was gently inserted into the vein. The catheters were tied with suture thread and held in position parallel to the veins. The catheters were then flushed with heparin-containing saline to maintain patency. One side of the catheter was used for glucose infusion, and the other side of the catheter was used for infusion of insulin, GLP-1 analog, or vehicle.

Infusion of Glucose and Test Compounds. Glucose solutions were prepared in normal saline each day prior to the start of the study. Exogenous glucose was infused as a constant infusion via the jugular vein at a variety of concentrations (as 10 or 20% solutions) starting at various times after anesthesia and catheterization. To achieve more rapid and sustained hyperglycemia, experiments were also performed in which initial bolus doses of glucose were administered followed by continuous infusion. Details of the concentration, volume, and time of infusions are described under Results. Blood samples were collected throughout the infusion period to determine blood glucose levels. Insulin was freshly prepared in phosphate-buffered saline (PBS) containing 0.01% Tween 20 prior to start of the experiment. GLP-1 analog was chemically synthesized, purified by reversed-phase chromatography on Vydac C18 columns, and lyophilized. The lyophilized peptide was dissolved in water, adjusted to pH 7.4, and frozen for storage at −10 to −20°C. Prior to use in animal studies, GLP-1 analog was diluted to the desired concentration in PBS containing 0.01% Tween 20. The infusion volume of insulin and GLP-1 analog was maintained constant for the duration of the experiment at a flow rate of 1 ml/kg/h, using a constant infusion pump (Harvard Apparatus Inc., Holliston, MA). Immediately following catheterization, the pump infused normal saline, which was replaced by vehicle or test substances as dictated by the experiment.

Determination of Glucoregulatory Hormones. To determine the plasma levels of different glucoregulatory hormones, blood samples were collected by tail bleed at several intermediate times and at the termination of the study (180 min). Terminal blood samples (5 ml) were collected in EDTA (0.5 M, pH 8.0; Invitrogen, Carlsbad, CA)-washed 10-ml syringes by direct cardiac puncture and transferred immediately into 5-ml Monoject blood collection tubes containing 7.5 mg of EDTA (Sherwood Medical, St. Louis, MO). The blood samples were then centrifuged at 2500g for 10 min, and plasma was collected following standard protocols established in our laboratory. Hormone levels were determined by using the Linco-Plex Kits (Linco Research Inc., St. Charles, MO).

Materials and Methods

General Procedure and Animal Maintenance. Experiments were carried out in male Sprague-Dawley rats weighing 200 to 250 g (Charles River Breeding Laboratories, Portage, MI). Following shipment, the rats were housed for at least 1 week to allow acclimatization prior to experimental studies. The animals were maintained on a regular 12-h light/dark cycle (6:00 PM to 6:00 AM) with food and water ad libitum unless otherwise noted. Experiments were performed in rats deprived of food for 24 h. The protocol used for this study was approved by the Animal Care and Use Committee of Eli Lilly and Company (Indianapolis, IN).

The animals were anesthetized with an i.m. injection of a combination of ketamine (100 mg/kg) and xylazine (5 mg/kg), which were
Drugs. The drugs used were ketamine hydrochloride injection (Fort Dodge Laboratories, Fort Dodge, IA) and xylazine injection (The Butler Company, Columbus, OH), insulin (Humulin), and GLP-1 analog (Eli Lilly and Company). Ketamine and xylazine were mixed prior to coadministration.

Statistical Analysis of Data. Statistical significance was determined based on analysis of covariance from SAS (version 8.2) statistical analysis program (SAS Institute, Cary, NC). Initial body weight and initial glucose were considered as baseline covariates for making adjustment of heterogeneity between animals. Box Cox or log transformation was applied to the parameters whose data distributions were skewed. For time-dependent effects of treatment in glucose data, repeated measure analysis with initial body weight and glucose as baseline covariates was applied. Data comparison was considered significant when the \( p < 0.05 \). Results are expressed as mean ± S.E.M.

Results

Effect of Anesthesia in Fed and Fasted Rats. Experiments were performed in both fed and fasted rats to study the effect of KX anesthesia on blood glucose levels. KX administered i.m. to fed rats resulted in a large increase in blood glucose levels constituting hyperglycemia (>150 mg/dl; Nauck et al., 2004) within 30 min and remaining elevated for 2 h. In contrast, KX did not produce frank hyperglycemia in 24-h fasted rats (Fig. 1). Since the anesthetic agent KX at this specific dose induced anesthesia but did not produce hyperglycemia in fasted rats, these conditions allowed for the development of a controlled model of acute hyperglycemia achieved by exogenous glucose infusion.

Effect of Continuous Intravenous Glucose Infusion. To study the induction of acute hyperglycemia by glucose administration, different concentrations of glucose were infused i.v. in KX-anesthetized fasted rats. Infusion of 10 and 20% glucose solution starting 30 min after induction of anesthesia resulted in elevation of blood glucose level that increased gradually but did not reach a plateau within the 3-h study period. As shown in Fig. 2, both concentrations of glucose produced a significant increase in blood glucose levels compared with saline infusion. We chose the 10% glucose infusion for subsequent experiments to identify conditions for more rapid onset.

Effect of Bolus Injection followed by Continuous Glucose Infusion. In subsequent experiments, rats received a bolus (<10 s) of varying volumes (0.2–0.6 ml) of 20% glucose just prior to the start of continuous infusion of 10% glucose solution. A dose-dependent increase in blood glucose level was observed with the 0.6-ml dose of 20% glucose producing the maximum acute increase in blood glucose level, which remained elevated for as long as the rats received the continuous glucose infusion (Fig. 3). In subsequent experiments, we determined whether continuous infusion of glucose was necessary to maintain sustained hyperglycemia. To explore this question, we fol-

![Fig. 1. Time-dependent effect of KX (100 mg/kg ketamine and 5 mg/kg xylazine i.m.) on blood glucose levels in fasting (closed circle) and fed (closed triangle) rats. Each point represents mean ± S.E.M., with number of animals given in parentheses. Zero time indicates preanesthesia values. ***, \( p < 0.001 \) versus fed rats.](#)

![Fig. 2. Effect of continuous infusion of glucose solution (10 and 20%) or saline on blood glucose levels in fasting animals. Glucose or saline infusion started at 30 min after anesthesia and continued for 150 min. Results are mean ± S.E.M., with number of animals given in parentheses. Zero time indicates preanesthesia values. **, \( p < 0.01 \); and ***, \( p < 0.001 \) versus saline group.](#)

![Fig. 3. Time-dependent effect of continuous infusion (10% glucose solution) following loading boluses of different volumes (0.2–0.6 ml of 20% glucose solution) in fasting animals. Continuous infusion with loading boluses produced increased blood glucose levels with quick onset and are bolus volume-dependent compared with continuous infusion only. Results are mean ± S.E.M., with number of animals given in parentheses. Zero time indicates preanesthesia values. All points beginning from 15 min after the onset of continuous prior to bolus (0.4–0.6 ml) glucose infusion are significantly \( (p < 0.05) \) higher than continuous infusion only. \( p \) values were not placed on each graph for clarity.](#)
Results are mean hyperglycemia, although at a slightly lower level with 30-min infusion. Continuous infusion for 30 or 150 min produced sustained lowering of the hyperglycemic response in a time-dependent manner that went below baseline levels, whereas GLP-1 analog (10 μg/kg/h i.v.) produced significant lowering of the hyperglycemic response but never went below baseline (Fig. 5).

**Effect of Insulin and GLP-1 Analog on Plasma Levels of Glucoregulatory Hormones.** Studies were performed to determine the effects of treatment with insulin and GLP-1 analog for 120 min on a variety of glucoregulatory and stress hormones including insulin, growth hormone, glucagon, adrenocorticotropic hormone, and corticosterone. Insulin did not result in changes in any of these hormones (Table 2). Similarly, GLP-1 analog had little effect on these glucoregulatory and stress hormones except that GLP-1 analog produced a significant increase in insulin levels compared with vehicle control (Table 2).

**Discussion**

The novel findings of this study are that suitable conditions were identified in which animals could be anesthetized allowing surgical intervention without manifestation of hyperglycemia, an animal model of acute sustained hyperglycemia with rapid onset in anesthetized rats has been developed, this novel model of controlled acute hyperglycemia may be used to evaluate the effect of acute hyperglycemia on disease induction and modification in anesthetized animals, and finally, the model was sensitive to glucose-lowering agents. Intramuscular injection of KX in fasted rats produced effective anesthesia without the hyperglycemia observed in fed rats. Under these conditions, rats receiving an i.v. infusion of glucose as a bolus followed by continuous infusion became hyperglycemic. This model can serve as an animal model in which the acute hyperglycemia can be induced at selected times. Furthermore, the model is sensitive to insulin and GLP-1 analog, which effectively lowered the elevated blood glucose to euglycemic levels.

In preclinical animal studies, the influence of hyperglycemia on stroke and myocardial infarction models has been performed mostly in diabetic animals with nondiabetic animals acting as control (Marfella et al., 2002; Shiomi et al., 2003) or in hyperglycemic animals in which glucose levels were raised by anesthesia or glucose infusion before induction of disease (Kawai et al., 1997). The present study thus aimed to identifying conditions where acute hyperglycemia could be induced after anesthesia and surgical maneuvers and institution of the disease model. Recently, we have re-

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![Fig. 4. Time-dependent effect of continuous infusion (10% glucose solution) following a loading bolus of 0.4 ml (20% glucose solution) in fasting animals. Continuous infusion for 30 or 150 min produced sustained hyperglycemia, although at a slightly lower level with 30-min infusion. Results are mean ± S.E.M., with number of animals given in parentheses. Zero time indicates preanesthesia values.](image-url)
ported that ketamine (100 mg/kg)/xylazine (10 mg/kg) and inhalation of isoflurane (2.5% in 1.5% oxygen) produce acute hyperglycemia in fed rats and did not produce hyperglycemia in fasted rats without the involvement of any surgical maneuvers (Saha et al., 2005b). In the present study, it has thus been shown that use of anesthetic agent KX with a low dose of xylazine (5 mg/kg) to fasted rats and further surgical maneuvers also did not result in hyperglycemia. Therefore, this approach was used to induce disease under euglycemic conditions.

It has been shown that infusion of a combination of glucose, insulin, adrenaline, and propranolol in 24-h fasted rats produced steady hyperglycemia, and this approach was used to study the effect of the glucose-lowering agent BTS 67582 (Page and Bailey, 1997). A later study (Ling et al., 2003) in fed rats has shown that continuous infusion of glucose raised blood glucose levels and that maintenance of steady-state hyperglycemia required constant glucose infusion. Recently, Takahashi et al. (2003) and Villafana et al. (2004) have reported that infusion of glucose for 30 min produced a short-lasting hyperglycemia in fasted rats. The blood glucose reached its peak at 30 min, at the end of the infusion period, and gradually returned back to baseline by 120 min (Takahashi et al., 2003). From all these studies, it is apparent that continuous glucose infusion is necessary to maintain sustained hyperglycemia. We could find no studies that describe conditions where hyperglycemia was induced rapidly and subsequently maintained for hours in the absence of glucose. Although studies have used glucose infusion-induced acute hyperglycemia (Kawai et al., 1997; Page and Bailey, 1997; Takahashi et al., 2003), no attempt has been made to clearly establish an animal model where sustained hyperglycemia may be achieved with short-term glucose infusion after anesthesia and surgical manipulations and/or disease induction. Such conditions would allow study of the influence of hyperglycemia after disease precipitation and also allow determination of sensitivity to glucose-lowering agents under hyperglycemic conditions. The present study determined that continuous infusion of glucose produced hyperglycemia but in a relatively slow fashion. Further studies with a loading bolus of glucose demonstrated that bolus doses (0.2–0.6 ml) of 20% glucose followed immediately by continuous infusion of glucose (10%) produced rapid and sustained hyperglycemia. Subsequent studies were performed to identify conditions where glucose could be infused for a short period of time. The present study clearly demonstrated that administration of a loading bolus of glucose (0.4 ml of 20% glucose) prior to continuous infusion of 10% glucose for 30 min can maintain a sustained hyperglycemic state for approximately 120 min. We believe that the conditions we identified will allow determination of the effect of acute hyperglycemia in animal models.

In the present study, we determined plasma levels of several glucoregulatory hormones following hyperglycemia. It was observed that among numerous glucoregulatory hormones measured, only glucagon levels were significantly decreased. The present study failed to detect any changes in insulin levels during hyperglycemia. Further studies are needed to more fully elucidate the biochemical consequence of glucose infusion-induced acute hyperglycemia in fasted rats.

Insulin produced significant correction of blood glucose levels in this glucose infusion-induced hyperglycemia model. The newly developed compound GLP-1 analog also showed efficacy similar to insulin in lowering blood glucose level in this model. GLP-1 analog has the advantage that it does not produce hypoglycemia as observed with insulin. The present rodent model has some clinical relevance and can be used in preclinical testing of compounds to treat hyperglycemia and hyperglycemia-associated illnesses. GLP-1 analog significantly increased insulin levels consistent with its known mechanism of action, namely stimulation of insulin release in

### Table 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vehicle (n = 12)</th>
<th>GLP-1 (n = 8)</th>
<th>Insulin (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (ng/ml)</td>
<td>0.25 ± 0.03</td>
<td>0.65 ± 0.26*</td>
<td>N.D.</td>
</tr>
<tr>
<td>GH (ng/dl)</td>
<td>45.02 ± 11.93</td>
<td>18.80 ± 3.04</td>
<td>36.20 ± 8.11</td>
</tr>
<tr>
<td>Glucagon (pg/ml)</td>
<td>73.91 ± 6.32</td>
<td>81.63 ± 10.82</td>
<td>160.25 ± 22.37**</td>
</tr>
<tr>
<td>Adrenocorticotrope hormone (pg/ml)</td>
<td>258.27 ± 56.71</td>
<td>238.88 ± 23.66</td>
<td>237.00 ± 49.69</td>
</tr>
<tr>
<td>Corticosterone (ng/ml)</td>
<td>299.45 ± 19.94</td>
<td>345.38 ± 26.88</td>
<td>358.50 ± 43.07</td>
</tr>
</tbody>
</table>

N.D., not determined.

* p < 0.05; ** p < 0.001 vs. vehicle.
the presence of hyperglycemia (Kjems et al., 2003; Vilsboll et al., 2003).

In conclusion, the present study demonstrated that in fasted rats, KX produces stable anesthesia and allows surgical manipulations without inducing hyperglycemia. Furthermore, we have identified a novel model that allows controlled induction of hyperglycemia in anesthetized nondiabetic animals. This will allow for the study of the influence of post-trauma hyperglycemia on lesion development and the examination of the impact of glucoregulatory intervention on pathology. Accordingly, we confirmed the ability of insulin and a GLP-1 analog to lower blood glucose levels during the hyperglycemic phase.

References


Ling PR, Mueller C, Smith RJ, and Bistrian BR (2003) Hyperglycemia induced by glucose infusion causes hepatic oxidative stress and systemic inflammation, but not STAT 3 or MAP kinase activation in liver in rats. Metabolism 52:868–874.


