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Anesthesia with propofol induces insulin resistance systemically in skeletal and cardiac muscles and liver of rats

Yoshikazu Yasuda, Yuji Fukushima, Masao Kaneki, J.A. Jeevendra Martyn *

Department of Anaesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, Shriners Hospitals for Children®, Harvard Medical School, Boston, MA 02114, United States

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ABSTRACT

Hyperglycemia together with hepatic and muscle insulin resistance are common features in critically ill patients, and these changes are associated with enhanced inflammatory response, increased susceptibility to infection, muscle wasting, and worsened prognosis. Tight blood glucose control by intensive insulin treatment may reduce the morbidity and mortality in intensive care units. Although some anesthetics have been shown to cause insulin resistance, it remains unknown how and in which tissues insulin resistance is induced by anesthetics. Moreover, the effects of propofol, a clinically relevant intravenous anesthetic, also used in the intensive care unit for sedation, on insulin sensitivity have not yet been investigated. Euglycemic hyperinsulinemic clamp study was performed in rats anesthetized with propofol and conscious unrestrained rats. To evaluate glucose uptake in tissues and hepatic glucose output [^{3}H]glucose and 2-deoxy[^{14}C]glucose were infused during the clamp study. Anesthesia with propofol induced a marked whole-body insulin resistance compared with conscious rats, as reflected by significantly decreased glucose infusion rate to maintain euglycemia. Insulin-stimulated tissue glucose uptake was decreased in skeletal muscle and heart, and hepatic glucose output was increased in propofol anesthetized rats. Anesthesia with propofol induces systemic insulin resistance along with decreases in insulin-stimulated glucose uptake in skeletal and heart muscle and attenuation of the insulin-mediated suppression of hepatic glucose output in rats.

1. Introduction

Insulin resistance is a common denominator in metabolic derangements associated with major surgery, trauma and critical illness (e.g., burn, sepsis) and is associated with hyperglycemia [1–4]. Hyperglycemia in critically ill patients is associated with increased mortality [5,6] and susceptibility to infection [7], polyneuropathy, renal insufficiency, and need for transfusion, increased ventilator-dependent days, intensive care unit (ICU) stay, and hospital stay, particularly in surgical patients [8,9]. Tight control of blood glucose levels by intensive insulin therapy has been shown to decrease the risk of wound infection, and reduce the morbidity and mortality in intensive care units [1,8,9], although controversial results were also reported [10,11], presumably at least in part due to increased incidence of severe hypoglycemia by intensive insulin therapy.

Earlier studies have demonstrated that pentobarbital induce whole-body insulin resistance in rats [12,13]. Previous studies have shown that volatile anesthetics, halothane, isoflurane and sevoflurane, impairs insulin secretion in rats, dogs and pigs [14–16], but the effects of these anesthetics on insulin sensitivity in tissues have not yet been investigated. Limited knowledge is, however, available how and in which tissues insulin resistance is induced by anesthetics. Propofol (2,6-diisopropylphenol) has been clinically used not only for induction and maintenance of anesthesia during surgical procedures but also for long-term sedation of patients in the ICUs [17,18]. Nonetheless, the effects of this widely used intravenous anesthetic, propofol, on insulin sensitivity have not yet been investigated.

Insulin resistance is defined as decreased responsiveness to metabolic actions of insulin, evidenced as decreased insulin-stimulated glucose uptake in tissues and enhanced hepatic glucose output [19]. Hyperinsulinemic euglycemic clamp technique, originally described by DeFronzo et al. [20], has been established as the gold standard to evaluate insulin sensitivity *in vivo* [19]. We therefore investigated the effects of anesthesia with propofol on whole-body insulin sensitivity, insulin-stimulated glucose uptake and hepatic glucose output by hyperinsulinemic euglycemic clamp study in rats.

* Corresponding author. Address: Harvard Medical School, Department of Anaesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, Shriners Hospitals for Children®–Boston, 51 Blossom Street, Room #206, Boston, MA 02114, United States. Fax: +1 617 371 4821.
E-mail address: jmartyn@partners.org (J.A.J. Martyn).

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2. Materials and methods

2.1. Animals

The Institutional Animal Care Committee approved the study protocol. The animal care facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Adult male Sprague–Dawley rats (220–280 g, Taconic Farms, Hudson, NY) were used for the study. The rats were housed in mesh cages in a room maintained at 25 °C and illuminated in 12:12-h light–dark cycles; they were provided with standard rodent chow and water *ad libitum*.

2.2. Hyperinsulinemic euglycemic clamp study

The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg kg^{-1}), and PE-50 catheters (Becton–Dickinson, Franklin Lakes, NJ) were implanted into the right jugular and femoral veins. The catheters were filled with saline containing heparin sodium. At 5–7 days after the catheter placement, hyperinsulinemic clamp study was performed as previously described (Fig. 1) [21]. Following overnight fasting, rats were randomly assigned to propofol anesthesia group and control conscious group. Anesthesia was induced by intravenous administration of propofol (a bolus injection of 10 mg kg^{-1} followed by constant infusion of 40 mg kg^{-1} h^{-1}) in propofol group, and same amount of saline was infused in control conscious unstrained rats. Rectal temperature was continuously monitored and maintained at 37 ± 0.5 °C by a heating pad (ATC 1000, World Precision Instruments, Sarasota, FL). At 15 min after the inception of propofol or saline infusion, the infusion of human regular insulin (Novolin R, Novo Nordisk Inc., Princeton, NJ) was initiated and maintained at the constant rate (20 mU kg^{-1} min^{-1}) via the jugular vein catheter. Blood samples were taken from the femoral vein catheter for the measurement of blood glucose levels at 5–10 min intervals. Blood glucose level was determined by the glucose oxidase method (Ascensia Contour Glucometer, Bayer, Tarrytown, NY). Euglycemia was maintained by varying the infusion rate of 50% glucose solution via the jugular vein catheter. Whole-body insulin sensitivity was evaluated by glucose infusion rate (GIR) that was required to maintain euglycemia at approximately 100 mg/dl during steady state (from 90 to 150 min after the inception of the insulin infusion). To evaluate hepatic glucose output, [^{3}H]glucose (6 μCi, American Radiolabeled Chemicals, St. Louis, MO) was administered by an intravenous bolus injection at the inception of insulin infusion, followed by constant infusion of [^{3}H]glucose (0.1 μCi/min). At 120 min after the initiation of insulin infusion, 2-deoxy[^{14}C] glucose (8 μCi, American Radiolabeled Chemicals) was administered by intravenous bolus injection to evaluate tissue glucose uptake. At 123, 125, 130, 140, and 150 min after the inception of insulin infusion, blood samples were collected to measure [^{3}H]glucose and 2-deoxy[^{14}C] glucose levels. At 150 min after the initiation of insulin infusion, the rats were euthanized with an overdose of pentobarbital (200 mg/kg ip) and then soleus muscle, gastrocnemius muscle, tibialis anterior muscle, rectus abdominis muscle, heart, liver, and epididymal fat were taken and frozen in liquid nitrogen immediately. All of the tissue samples were stored at −80 °C until assayed.

2.3. Measurement of radiolabeled glucose concentrations in plasma and tissues

To determine the radioactivity of [^{3}H]glucose and 2-deoxy[^{14}C] glucose in blood, aliquots of plasma (50 μl) obtained with heparin sodium were deproteinized with 100 μl of $Ba(OH)_2$ (0.3 N) and $ZnSO_4$ (0.3 N) and then centrifuged, as previously described [21]. The radioactivity of ^{3}H and ^{14}C in the protein-free supernatants of $Ba(OH)_2$ and $ZnSO_4$ precipitates was measured with a liquid scintillation counter. To eliminate tritiated water, the supernatants were evaporated to dryness for the measurement of [^{3}H]glucose. Plasma glucose specific activities were calculated by dividing ^{3}H and ^{14}C radioactivities in the dried, reconstituted plasma samples by ambient plasma glucose concentration. To measure the radioactivity of 2-deoxy[^{14}C]glucose in tissues, the tissue samples (200 mg) were digested with 0.5 ml of 1 M NaOH at 60 °C for 1 h and then neutralized with 0.5 ml of 1 M HCl. Aliquots of 200 μl of digested tissue samples were mixed with 1 ml $HClO_4$ (4% wt./vol) and then centrifuged. Thereafter, the radioactivity of ^{14}C in the supernatants was measured [21]. Tissue glucose uptake (Rg) was calculated as follows [22].

$$R_g(\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = C_p \times C^*_m / \int_{120}^{150} C^*_p(t)dt$$

Where C_p is the steady-state plasma glucose concentration, C^*_m is the tissue accumulation of 2-deoxy[^{14}C] glucose per unit mass, and $C^*_p(t)$ is the plasma 2-deoxy[^{14}C] glucose concentration.

2.4. Calculation of hepatic glucose output

Hepatic glucose output was determined as previously described [23]. Briefly, glucose appearance rate was calculated as the ratio of the infusion rate of ^{3}H and the steady-state plasma [^{3}H]glucose specific activity. The rate of hepatic glucose output was calculated as glucose appearance rate minus the glucose infusion rate

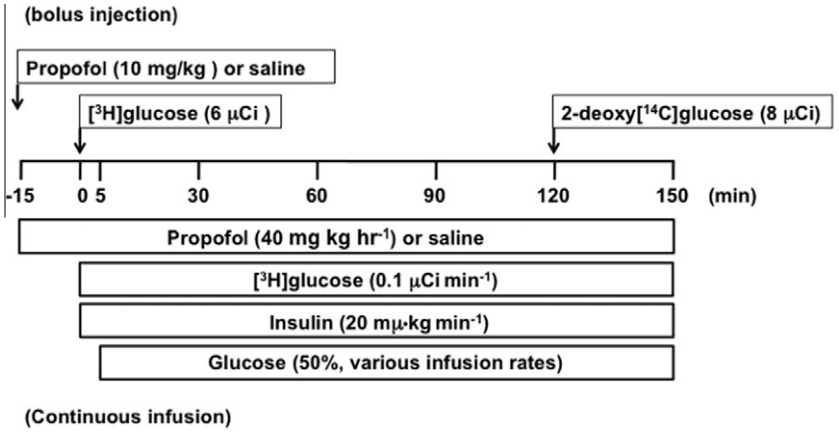

Fig. 1. Experimental protocol of hyperinsulinemic euglycemic clamp study.

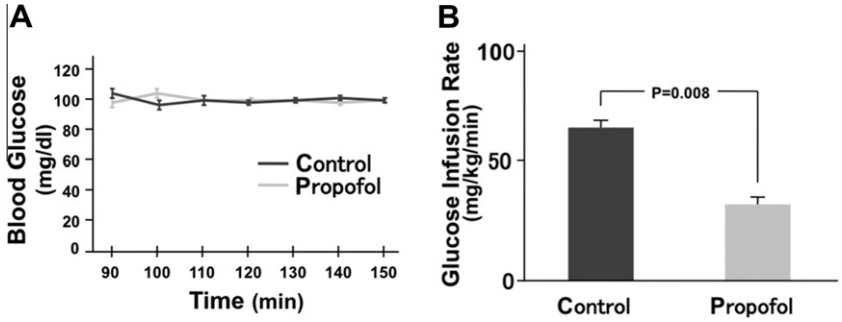

Fig. 2. Blood glucose levels during the steady-state period of hyperinsulinemic euglycemic clamp study and the effect of propofol on glucose infusion rate (GIR). (A) blood glucose levels did not differ between propofol anesthesia and control conscious groups. $n = 8$ for each group, and (B) Glucose infusion rate to maintain euglycemia was significantly greater in control conscious rats than propofol anesthetized rats. $n = 8$ for each group. The lower GIR during propofol infusion to maintain euglycemia suggests systemic insulin resistance.

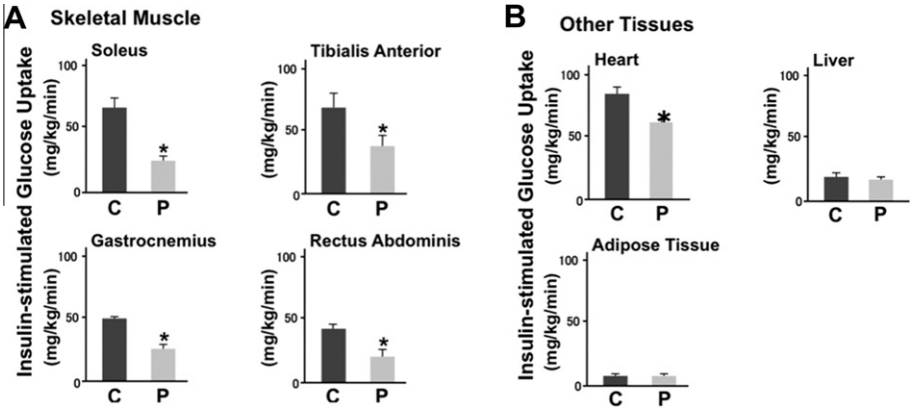

Fig. 3. Effects of propofol on insulin-stimulated glucose uptake in tissues. Insulin-stimulated glucose uptake was decreased by anesthesia with propofol (P) compared to conscious rats (C) in skeletal muscles (soleus, tibialis, anterior, gastrocnemius, and rectus abdominis) (A), and heart (B). However, no difference was found in insulin-stimulated glucose uptake in liver and adipose tissue (B). *P, 0.05 vs. conscious rats, $n = 4$ for each group.

(hepatic glucose output = glucose appearance rate – glucose infusion rate).

2.5. Statistical analysis

The data were compared using Mann–Whitney U test. The null hypothesis was rejected when $P < 0.05$. All values are expressed as means ± SEM.

3. Results

3.1. Effects of anesthesia with propofol on glucose infusion rate during euglycemic hyperinsulinemic clamp

Euglycemic hyperinsulinemic clamp study was performed to examine the effects of anesthesia with propofol on insulin sensitivity. Body weights did not differ between propofol and control groups (propofol: 254.8 ± 6.8, control: 246.3 ± 4.8 g). Blood glucose levels also did not differ between propofol and control groups during the steady-state period (Fig. 2A). The average blood glucose levels during the steady state were 101.9 ± 2.0 and 101.1 ± 2.4 mg/dl in propofol and control groups, respectively.

Anesthesia with propofol induced a marked whole-body insulin resistance compared with conscious rats, as reflected by significantly decreased GIR. The GIR during the euglycemic hyperinsulinemic clamp were 174.8 ± 12.2 and 343.6 ± 10.5 μmol kg^{-1} min^{-1} in the propofol and control groups, respectively (Fig. 2B). Insulin-stimulated glucose uptake by skeletal muscle (Fig. 3A) and heart (Fig. 3B) during the glycemic hyperinsulinemic clamp study was significantly decreased by anesthesia with propofol, as compared with conscious rats. Insulin-stimulated glucose uptake was reduced to 35% in soleus, 53% in tibialis anterior, 52% in gastrocnemius, 52% in rectus abdominis, and 74% in heart of propofol anesthetized animals compared to those in conscious animals. On the other hand, insulin-stimulated glucose uptake in the liver (propofol: 88.1 ± 11.4, control: 94.5 ± 15.8 μmol kg^{-1} min^{-1}) and adipose tissue (propofol: 31.7 ± 3.0, control: 27.3 ± 3.2 μmol kg^{-1} - min^{-1}) did not significantly differ between propofol and control

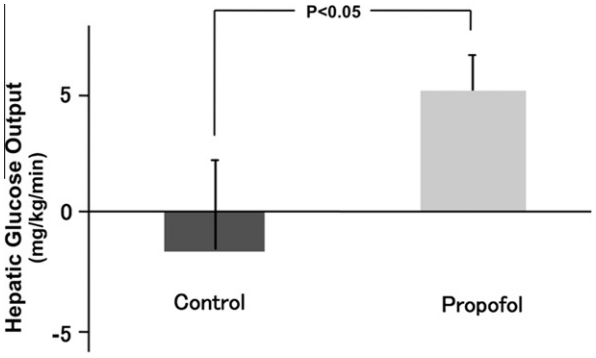

Fig. 4. Effect of propofol on hepatic glucose output. Anesthesia with propofol significantly increased hepatic glucose output during the hyperinsulinemic euglycemic clamp, as compared with control conscious rats. $n = 4$ for each group.

groups (Fig 4B). Hepatic glucose output was significantly greater in anesthetized rats (27.5 ± 8.6 μmol kg^{-1} min^{-1}) than conscious rats (−8.3 ± 20.4 μmol kg^{-1} min^{-1}, $P < 0.05$) (Fig. 4).

4. Discussion

This study demonstrates that anesthesia with propofol resulted in whole-body insulin resistance, as judged by the decreased glucose infusion rate (GIR) during euglycemic hyperinsulinemic clamp (Fig. 2B), attenuated insulin-stimulated glucose uptake in skeletal muscle and heart (Fig. 3A and B), and attenuated insulin-mediated inhibition of hepatic glucose output, as compared with conscious rats (Fig. 4). Our results indicate that attenuated insulin-stimulated glucose uptake in muscle and impaired insulin-mediated inhibition of hepatic glucose output are involved in propofol-induced systemic insulin resistance.

Insulin is the principal hormone in the maintenance of glucose homeostasis. It stimulates glucose uptake into muscle, glycogen synthesis in the liver and muscle, and fat deposition in adipocytes [24,25]. Other important actions of insulin include promotion of protein synthesis, cell survival, cell growth, inhibition of protein degradation, and anti-inflammatory effects [26–29]. Thus, insulin resistance exacerbates muscle catabolism and wasting, increases susceptibility to infection, and enhances inflammation, even in the absence of overt hyperglycemia in critical illness [30].

Recently, the development of muscle wasting in critical illness is increasingly recognized as a cause of failure to wean from mechanical ventilation and is associated with prolonged rehabilitation and hospitalization, and increased morbidity and mortality [31–33]. Increased risk of muscle wasting in critically ill patients has been shown to be related to elevated plasma glucose levels, and intensive insulin therapy can decrease the incidence of myopathy [31–33]. One can reasonably speculate, therefore, that propofol-induced insulin resistance might affect the prognosis of critically ill patients not only by promoting hyperglycemia but also by enhancing catabolism and muscle wasting, particularly during long-term infusion.

Prolonged (>48 h) propofol administration at high doses (>4 mg/kg/h) may cause a rare, but frequently fatal complication known as propofol infusion syndrome (PRIS) [34–35]. PRIS is characterized by metabolic acidosis, rhabdomyolysis of both skeletal and cardiac muscle, arrhythmias, myocardial failure, renal failure, hepatomegaly and death [34–36]. Although the mechanisms responsible for PRIS have not been fully understood, mitochondrial dysfunction, including electron transport and fatty acid oxidation, has been proposed to underlie PRIS [36–40]. Propofol also causes glycogen synthase kinase-3β-related mitochondrial dysfunction and apoptosis [41]. A close biological link between mitochondrial dysfunction and insulin resistance has been documented [42–44]. Insulin resistance is associated with impaired mitochondrial electron transport and fatty acid oxidation in skeletal muscle and heart [45,46]. It is possible, therefore, that propofol-induced insulin resistance might contribute to the development of PRIS. Further studies are required to clarify this point.

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