Anesthesia rapidly suppresses insulin pulse mass but enhances the orderliness of insulin secretory process

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Vore, Stephen J., E. Dale Aycock, Johannes D. Veldhuis, and Peter C. Butler. Anesthesia rapidly suppresses insulin pulse mass but enhances the orderliness of insulin secretory process. Am J Physiol Endocrinol Metab 281: E93–E99, 2001.—Induction of anesthesia is accompanied by modest hyperglycemia and a decreased plasma insulin concentration. Most insulin is secreted in discrete pulses occurring at ~6- to 8-min intervals. We sought to test the hypothesis that anesthesia inhibits insulin release by disrupting pulsatile insulin secretion in a canine model by use of direct portal vein sampling. We report that induction of anesthesia causes an abrupt decrease in the insulin secretion rate (1.1 ± 0.2 vs. 0.7 ± 0.1 pmol·kg⁻¹·min⁻¹, P < 0.05) by suppressing insulin pulse mass (630 ± 121 vs. 270 ± 31 pmol, P < 0.01). Anesthesia also elicited an ~30% higher increase in insulin pulse frequency (P < 0.01) and more orderly insulin concentration profiles (P < 0.01). These effects were evoked by either sodium thiamylal or nitrous oxide and isoflurane. In conclusion, anesthesia represses insulin secretion through the mechanism of a twofold blunting of pulse mass despite an increase in orderly pulse frequency. These data thus unveil independent amplitude and frequency controls of β-cells’ secretory activity in vivo.

INDUCTION OF ANESTHESIA is accompanied by a small increase in the plasma glucose concentration (2, 7). Indeed, numerous drugs used for induction of anesthesia lead to suppression of insulin secretion (1, 5, 8, 9, 14, 33, 38, 39). Although it has been known for many years that insulin is secreted in a pulsatile manner (11, 18), recent studies show that the majority of insulin secreted is derived from discrete insulin secretory bursts generated approximately every 6–8 min (29). Regulation of insulin secretion is achieved by modulation of the mass of insulin secretory bursts (26–28). Validated methods allow qualification of pulsatile insulin secretion and the orderliness of insulin secretion in vivo (29). Impaired pulsatile insulin secretion is an early marker for patients at risk of developing type 1 or type 2 diabetes (4, 21). Increased entropy (pattern irregularity) in insulin concentration profiles also emerges in humans at risk of type 2 diabetes (34). Insights into the mechanisms by which induction of anesthesia influences pulsatile insulin secretion may shed light on the genesis of pulsatile insulin secretion as well as provide clinically relevant information. Experimentally, anesthesia is commonly used during research studies in animals when altered insulin secretion may be an important confounding variable. Therefore, a clear understanding of the effects of anesthesia on insulin secretion in vivo is important clinically and experimentally.

Here we sought to establish the impact of induction of anesthesia on insulin secretion in vivo. We postulated that anesthesia disrupts the pulsatile component of insulin secretion. We also examined how induction of anesthesia influences the orderliness of the insulin release process, the mechanisms of which are poorly understood. To this end, we used a validated canine model (29) with chronic portal vein catheterization to sample portal venous blood directly to capture insulin secretory profiles before and after induction of anesthesia.

MATERIALS AND METHODS

The present studies were approved by the East Carolina University Institutional Animal Care and Use Committee and conducted in accordance with the Animal Welfare Act and Institute of Laboratory Animal Research (“Guide for the Care and Use of Laboratory Animals”) rules and guidelines. Eight dogs (mixed breed, 20–30 kg) were studied.

Surgical Implantation of Protocol Vein Catheters

The surgical implantation of the portal vein sampling catheters has been described in detail (29). In brief, with the animal under general anesthesia, one end of a 50-cm-long, 0.040-mm-ID × 0.085-mm-OD medical grade Silastic catheter (Dow Corning Medical Products, Midland, MI) with a
0.078-mm-ID × 0.125-mm-OD cuff 4 cm from the end was inserted into the portal vein and its tip advanced to the bifurcation of the portal vein into hepatic veins. The catheter was secured above the cuff with the purse string. The catheter was then passed through a blunt incision in the muscle wall at the level of the diaphragm high on the right side and tunneled to a subcutaneous pocket between the scapulae.

Animals received cefazolin (22 mg/kg iv Kezol, Eli Lilly, Indianapolis, IN) intraoperatively. During the immediate postoperative period after recovery from anesthesia, oxymorphone (Numorphan, Du Pont Pharmaceuticals, Monati, PR) was administered at a dose of 0.023–0.22 mg/kg intramuscularly every 3 h or as needed for the first 12 h, and animals were closely watched. Additional analgesia was provided as necessary. Depo-penicillin, a long-acting benzathine/procaine penicillin (30,000 U/kg im Durapen; VEDCO, Overland Park, KS), was administered every other day for 1 wk as a prophylaxis. All studies were performed ≥1 wk postoperatively when dogs were eating all their food rations and had normal bowel actions. Dogs were accustomed to the study personnel and the laboratory sling before the study.

**Study Protocol 1**

After an overnight fast, animals (n = 5) were placed in a laboratory dog sling (Alice King Catham Medical Arts, Los Angeles, CA), and the portal vein sampling catheter was exteriorized from the subcutaneous pocket under local anesthesia with 2% lidocaine (Astra Pharmaceutical, Westborough, MA). An 18-gauge intravenous sampling catheter (Jelco, Critikon, Tampa, FL) was also placed in the cephalic vein of one foreleg to provide access for peripheral sampling. A similar intravenous catheter was placed in the contralateral leg for administration of saline (0.9%) at 50 ml/h for the duration of the study. Blood samples (1.5 ml) were collected simultaneously from both catheters at 1-min intervals for 60 min (t = 0–60 min) and placed in iced EDTA tubes for subsequent insulin assay. A 1.0-ml blood sample was obtained from the peripheral catheter every 6 min and placed in iced potassium oxalate-sodium fluoride tubes for glucose assays. At t = 60 min, sampling was suspended for 15 min, and both catheters were heparin-locked while anesthesia was induced with sodium thiamylal as in protocol 1, but isoflurane and nitrous oxide were not administered until t = 120 min; these were continued until the end of the extended sampling period of t = 240 min. During the sampling period, 60- to 120-min anesthesia was maintained by administration of additional small doses of sodium thiamyl as necessary. The purposes of these modifications were 1) to assess whether the effect of induction of anesthesia on pulsatile insulin secretion was immediate, and 2) to attempt to factor out whether the barbiturate induction and/or the volatile anesthetic gases were responsible for disruption of pulsatile insulin secretion.

**Assays**

Plasma insulin concentrations were measured in duplicate as previously described (29). Glucose concentrations were determined with a Genstar II analyzer (Electronucleonics, Fairfield, NJ) with a hexokinase enzymatic methodology.

**Calculations and Statistical Analysis**

Portal vein insulin concentration profiles were submitted to deconvolution analysis, as previously described (29), to determine the location and magnitude of insulin secretory bursts. The resulting data are expressed as mass units of insulin secreted per unit volume of distribution per unit of time. The frequency and mass of insulin secretory bursts in each dog were compared before and after induction of anesthesia by use of a paired Student’s t-test. Orderliness of insulin concentration profiles was calculated with the model-independent statistic approximate entropy (ApEn) (23, 24).

**RESULTS**

**Protocol 1**

**Portal vein sampling.** The mean plasma glucose concentration remained stable before anesthesia and was not significantly altered after induction of anesthesia (Fig. 1). In contrast, the mean peripheral and portal vein plasma insulin concentration decreased precipitously after induction of anesthesia (P < 0.01), with partial recovery thereafter. Portal vein plasma flow decreased 37% after induction of anesthesia.

Inspection of the portal vein insulin concentration profile in each dog showed consistent patterns (Fig. 2). Before anesthesia, discrete oscillations in insulin concentration occurred at ~7-min intervals, as previously reported (26–29). After induction of anesthesia, the amplitude of insulin oscillations decreased in all dogs (630 ± 121 vs. 270 ± 31, P < 0.05), showing partial recovery ~1 h after induction of anesthesia. Deconvolution of the plasma insulin concentration profiles disclosed that induction of anesthesia inhibited insulin secretion (1.1 ± 0.2 vs. 0.7 ± 0.1 pmol·kg⁻¹·min⁻¹, P < 0.05) through the mechanism of suppression of the mean insulin secretory pulse mass (277 ± 34 vs. 145 ± 17 pmol, P < 0.01). Coincidentally, there was an ~30% increase in pulse frequency (7.2 ± 0.4 vs. 5.2 ± 0.3, P < 0.01; Figs. 3 and 4). ApEn decreased in all dogs after induction of anesthesia (P < 0.01; 1.23 ± 0.08 vs.
Peripheral vein insulin concentrations (bottom) in the basal fasting state (0–60 min) and after induction and maintenance of anesthesia at t = 60 min with sodium thiamylal and nitrous oxide and isoflurane, respectively.

Peripheral sampling. The insulin concentration profile obtained from concurrent peripheral vein sampling exhibited the same pattern as that in the portal vein. Thus a marked decrease in insulin burst mass \((P < 0.01)\) and a lesser increase in pulse frequency \((P < 0.05)\) were observed in response to induction of anesthesia.

Protocol 2

As in protocol 1, the plasma glucose concentrations remained stable before induction of anesthesia but increased slightly after induction before returning to baseline. As anticipated by results from protocol 1, the portal vein insulin concentration profile showed distinct oscillations occurring approximately once every 6 min. Pulses were suppressed immediately on induction of anesthesia by barbiturate alone (Fig. 5). During anesthesia with barbiturate only \((t = 60–120 \text{ min})\), insulin oscillations remained damped. During anesthesia with volatile anesthetic gases only \((t = 120–240 \text{ min})\), pulsatile insulin secretion remained fully suppressed in two of the dogs but showed some late recovery similar to that observed in some dogs in protocol 1. Deconvolution of these data again revealed that the frequency of pulsatile insulin secretion increased slightly after induction of anesthesia with either barbiturate or volatile gas compared with baseline, and the mass of insulin secretory bursts fell during anesthesia.

DISCUSSION

In the present study, we tested the hypothesis that induction of anesthesia inhibits insulin secretion by disrupting the pulsatile component of insulin release. We report that induction of anesthesia causes an immediate and profound repression of insulin secretion and that the mechanism of this effect is selective inhibition (~2-fold) of insulin secretory burst mass. Suppression of insulin pulse mass occurred when barbiturate only was used to induce anesthesia and was retained during anesthesia with isoflurane and nitrous oxide. Furthermore, induction of anesthesia enhanced the orderliness of serial insulin concentration profiles and led to an ~30% increase in pulse frequency.

Induction of anesthesia can be accompanied by a transient decrease in the plasma insulin concentration in the systemic circulation (2, 7). Glucose-mediated insulin secretion also is impaired after induction of anesthesia (1, 5, 8, 9, 14, 33, 38, 39). However, other
investigators have reported that the basal plasma insulin concentration is not changed after induction of anesthesia (17). This discrepancy may reflect in part the transient nature of change in systemic insulin concentrations (Fig. 1). e.g., if a secondary rise in the plasma glucose concentration leads to a compensatory increase in the rate of insulin secretion despite anesthesia. Alternatively, the anesthesia-related decrease in insulin pulse mass may result in decreased hepatic insulin clearance. We have previously reported that a low-dose somatostatin infusion, which causes damping of insulin oscillations comparable to those observed in the current study, is associated with decreased hepatic insulin clearance (28). However, in a prior study in dogs, the hepatic insulin clearance rate was similar after a glucose bolus in anesthetized and awake dogs (17). This observation is consistent with our findings during low-dose somatostatin infusion after glucose ingestion, wherein glucose-enhanced amplification of insulin oscillations restored hepatic insulin clearance (28).

The exact mechanism by which the induction of anesthesia inhibits the amount of insulin secreted per burst and, conversely, elevates insulin pulse frequency remains unclear. Coordinate pulsatile insulin release requires a pacemaker and islet-to-islet coordination. Within the pancreas, the nature of either of these synchronizing mechanisms remains largely unknown, although individual islets have automaticity, and each islet is therefore a potential pacemaker (3, 6). Islet-to-

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**Fig. 3.** Insulin secretion profiles in the portal vein of the 2 representative dogs shown in Fig. 2 (protocol 1) in the basal fasting state (t = 0–60 min) and after induction of anesthesia at t = 60 min. The magnitude of insulin pulses falls after induction of anesthesia, but their frequency increases.

**Fig. 4.** Insulin pulse mass (left), interpulse interval (middle), and orderliness of insulin release (right) in dogs, assessed by portal vein sampling in protocol 1 both before and after induction of anesthesia. As a measure of orderliness of the secretory process, higher approximate entropy (ApEn) denotes decreased orderliness (and vice versa). The vertical scale is inverted to highlight this relationship.
paired insulin secretion in response to a glucose bolus in humans (13), presumably as a consequence of the loss of β-adrenergic-mediated augmentation of insulin release (31). Induction of anesthesia can also transiently inhibit and then stimulate β-adrenergic activity (32). Although stimulation of α-adrenergic receptors by epinephrine has been shown to inhibit insulin secretion (30), many anesthetic agents inhibit the α-adrenergic system (19). Furthermore, anesthetic agents inhibit insulin release from in vitro preparations of islet material (10, 22), which suggests that anesthesia has direct actions at the level of the β-cell. Also, inhalation-induced anesthesia without surgery leads to inhibition of insulin secretion with either no change or a decrease in circulating catecholamine levels (13), suggesting that inhibition of anesthesia may be independent of the sympathetic system.

An interesting aspect of this study is that the inhibition of pulsatile insulin secretion after induction of anesthesia was not accompanied by any change in plasma glucose concentration. One interpretation of these findings might be that pulsatile insulin secretion is not important in the regulation of glucose metabolism. Prior studies in which anesthesia was induced without surgery also show little or no change in plasma glucose concentration (13, 16), whereas those accompanied by surgery generally reveal an increase in plasma glucose concentration (1). In a prior study of the metabolic consequences of induction of anesthesia in dogs, Horber et al. (14) also reported no change in plasma glucose concentration. In a prior study of the metabolic consequences of induction of anesthesia in dogs, Horber et al. (14) also reported no change in plasma glucose concentration. Horber et al. also measured glucose turnover and reported that induction of anesthesia was followed by an increase in hepatic glucose release that was offset by a simultaneous increase in glucose disposal. From their data, it is tempting to speculate that the decline in portal vein insulin pulses delivered to the liver after anesthesia contributes to the increase in hepatic glucose release, whereas separate processes that may include direct actions of anesthesia on muscle lead to the decrease in glucose uptake by peripheral tissues. The complexity of interpreting the relationship between changes in insulin secretion and other parameters of metabolism is emphasized by the fact that Horber et al. also reported that induction of anesthesia resulted in an abrupt decrease in fatty acid turnover and a decrease in the rate of leucine turnover, leucine oxidation, and leucine incorporation into proteins, whereas overall leucine catabolism (ratio of leucine oxidation to nonoxidative leucine disappearance) decreased. The authors concluded that these data were best explained by widespread direct actions of anesthesia on numerous different aspects of metabolism in multiple tissues.

The observation that induction of anesthesia evokes enhanced orderliness (decreased entropy) of serial plasma insulin release could suggest that anesthesia reduces the number or strength of regulatory inputs on insulin secretion. For example, anesthesia may restrict sympathetic inputs. Anesthetic agents also tend to

![Graph](https://via.placeholder.com/150)

**Fig. 5.** Portal vein insulin concentration profiles in the 3 dogs in protocol 2, shown in the basal fasting state (t = 0–60 min) during anesthesia with sodium thiamylal only (t = 60–120 min) and during anesthesia with isoflurane and nitrous oxide only (t = 120–140 min).
stabilize cell membrane electrical activity. Stabilization of islet-cell secretory responses to the same number of regulatory input signals would, in principle, result in a more orderly pattern of hormone release, because the orderliness of neurohormone output is controlled jointly by the effective strength and multiplicity of the network (23, 24).

In conclusion, induction of anesthesia causes a prompt inhibition of insulin secretion that is mechanistically achieved by selective suppression of the insulin secretory burst mass, resulting in decreased amplitude of insulin oscillations. Use of a validated deconvolution technique further indicates that induction of anesthesia elicits an ~30% increase in pulse frequency and enhances the orderliness of the serial insulin release process. These findings in ensemble point to independent islet-cell effects of anesthesia on the amplitude and frequency control of pulsatile insulin secretion, thereby unmasking a dissociation in the amplitude and frequency-dependent modulation of β-cell secretory function.

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