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 thiamyal 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 control of B-cells' secretory activity in vivo.

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 pump 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, Monaté, PR) was administered at a dose of 0.023–0.22 mg/kg intramuscularly every 3 h 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 (17 mg/kg Biotal; Boehringer Ingelheim Animal Health, St. Joseph, MO). Anesthesia was maintained with isoflurane (0.5–1.0%; Forane, Anaquest, Ingelheim Animal Health, St. Joseph, MO). Anesthesia was induced with sodium thiamylal (17 mg/kg Biotal; Boehringer Ingelheim Animal Health, St. Joseph, MO). Anesthesia was maintained with isoflurane (0.5–1.0%; Forane, Anaquest, Ingelheim Animal Health, St. Joseph, MO). Anesthesia was induced with sodium thiamylal (17 mg/kg Biotal; Boehringer Ingelheim Animal Health, St. Joseph, MO). Anesthesia was maintained with isoflurane (0.5–1.0%; Forane, Anaquest, Ingelheim Animal Health, St. Joseph, MO).

Inspection of the portal vein insulin concentration sampling period, with the dog conscious in a laboratory sling (t = 0–60 min), portal vein sampling was continued at the time of anesthesia induction for 15 min. Second, 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 thiamylal 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·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 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 (about 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 \(\sim 30\%\) 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-islet...
ANESTHESIA ALTERS INSULIN SECRETION

E97

islet coordination appears to require intraorgan neural interconnections in vivo (25). Theoretically, induction of anesthesia may disrupt pulsatile insulin release either through effects on the putative pacemaker or by disrupting the mechanism that facilitates islet-to-islet coordination. Extrinsic denervation of the pancreas, which occurs in an isolated perfused pancreas (36, 37) or a transplanted pancreas (20, 35), does not ablate and appears to increase the frequency of coordinate pulsatile insulin secretion (35) (analogous to the denervated heart). However, the intrinsic intraorgan neural network also appears to be important (analogous to Purkinje tissue), as intrahepatic transplanted islets in diabetic rats only achieve coordinate pulsatile insulin release once reinnervated by the intrahepatic neural network (25). Coordinate pulsatile insulin release from the isolated perfused livers of those rats was unaffected by acute exposure to the barbiturate pentobarbital (25). Although central nervous system input to the pancreas is not required to generate pulsatile insulin secretion, it is plausible that the inhibition of pulsatile insulin release after induction of anesthesia is mediated by the inhibitory effect of an extrinsic neural input such as sympathetic neural activity. Sympathetic blockade by spinal anesthesia results in impaired 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, despite a decrease in peripheral insulin concentration in response to anesthesia. 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
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 $b$-cell secretory function.

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