学位論文内容の要約
Dissertation Summary

学位論文題目

Measurement and analysis of blood glucose levels in rats at 5, 10, and 15 minutes after administration of general anesthetics.

（全身麻酔薬投与後 **5, 10, 15** 分における ラットの血糖値の測定と解析）

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In recent years, from the perspective of animal ethics, appropriate analgesic effects have been required in the use of anesthesia for laboratory animals. Pentobarbital anesthesia (Nembutal®) was commonly used for rat surgeries; however, since this drug does not provide analgesic effects, it has been recommended to avoid using it alone since around 2016 (Dikmen et al, 2016; Okamura, 2016). Then, a new anesthesia method—a mixture of three drugs (MMB, medetomidine hydrochloride 0.375, midazolam 2, and butorphanol tartrate 2.5 in mg/kg) has become widely adopted worldwide. MMB has been demonstrated to provide adequate sedation, analgesia, and muscle relaxation as proper anesthetic effects, and its impact on vital signs and the composition of blood and body fluids has also been examined. Since MMB contains medetomidine, an α2-adrenergic receptor agonist, its effects on glucose metabolism and insulin secretion regulation via α2-adrenergic receptors have been reported, and caution has been advised when using MMB in diabetic model rats (Kanda & Hikasa, 2008a; Kanda & Hikasa, 2008b; Guedes & Rude, 2013; Tokunaga, 2021).

MMB is known to markedly increase blood glucose levels (Okamura, 2016). The inhalation anesthetics isoflurane (ISO) and sevoflurane (SEVO), which are also commonly used in animal experiments, have a blood glucose-elevating effect, although not as much as MMB (Dikmen et al, 2016; Okamura, 2016). On the other hand, propofol and pentobarbital (Nembutal®, Somnopentyl®, now discontinued), which should not be applied alone, have no glycemic elevating effect (Kitamura et al, 2009; Okamura, 2016). These data were measured at 15 minutes or more after anesthesia, so the acute changes in blood glucose levels within the first 15 minutes after anesthesia are unknown.

We conducted blood sampling from the tail vein under MMB anesthesia to use blood glucose levels as an indicator of hunger in rats kept under various fasting and feeding schedules. In fact, when we measured blood glucose levels in blood samples acutely obtained from rats within 10 min after MMB injection, we found that they were significantly higher than normal, but there was no previous study that measured such acute blood glucose elevation occurred within minutes after anesthesia administration. So, there was no knowledge to explain the mechanism of acute blood glucose elevation at all. Therefore, the aim of this study was to investigate the temporal changes in blood glucose levels immediately after the administration of anesthesia and to clarify the mechanism of acute hyperglycemia induced by general anesthetics.

Thirty-six male Sprague-Dawley rats, seven weeks of age at the start of the study, were used for the experiments. Six or 5 rats were assigned to each experimental group, and analysis was performed when data were obtained from five or more rats in each group. Rats were individually kept in cages, and the light/dark cycle was 12h. During the fasting and water deprivation schedule, a wire mesh floor was used instead of paper bedding. Rats were feed by normal diet and distilled water. All animals were handled according to the Hokkaido University Animal Experiment Guidelines.

To enable blood collection by puncturing the tail vein of unanesthetized rats with an 18G injection needle, it is necessary to restrain the rat's body during sampling. Therefore, every blood collection was performed using a homemade device to immobilize the rat's movements. Blood samples were collected from the tail vein at 5, 10 and 15 minutes after administration of general anesthetics. We examined the effect of MMB containing midazolam (MID, Midazolam®, FUJIFILM Wako Chemicals Co., Ltd., Japan; 2 mg/kg i.p.), butorphanol (BT, Vetorphale®, Meiji Animal Health Co., Ltd., Japan; 2.5 mg/kg i.p.), and medetomidine

hydrochloride (MED, Medetomin®, Meiji Animal Health Co., Ltd., Japan; 0.375 mg/kg i.p.), single administration of each component of MMB, propofol (PROP, 1%, 20mL ampule, Viatris Inc. 10 mg/kg i.v. or i.p.), Isoflurane (ISO, 3% for 1 min and 2% for 14 min), Sevoflurane (SEVO, 3% for 1 min and 2% for 14 min) and saline (1mL/kg BW i.p. or i.v.). Immediately after blood collection, blood glucose levels of the collected blood samples were measured using a blood glucose meter for laboratory animals (Required specimen volume 1.1μL, Folacare Japan, Inc.). The same blood glucose measurement experiments were also performed on unanesthetized, restrained rats. To standardize the effects of feeding and drinking on changes in blood glucose levels, a fasting and water deprivation schedule as shown in Fig. 2 was used. Most rats underwent a 22-hour fasting period from 11:00 a.m. to 9:00 a.m. the following day, followed by a one-hour period with free access to food and water. After this, they experienced another one-hour fasting and water deprivation period before starting the blood glucose measurement experiment. Additionally, some rats had an extra twohour fasting and water deprivation period after the initial 22 hours, making a total of 24 hours of fasting, after which the blood glucose measurement experiment was started immediately.

Blood glucose values of 5 measurements (2 measurements before the administration of anesthetics and 3 measurements at 5, 10, 15 min after the administration of anesthetics) were compared using one-way ANOVA to assess effects of treatment. When overall significance was detected, post-hoc comparisons were conducted using Tukey's test. To compare the change rate of the blood glucose level at 5, 10, 15 min after the administration of anesthetics against the averaged value of 2 measurements before the administration of anesthetics, we used one-way ANOVA with Dunnett's test. Data are expressed as mean ± SEM. Significance was defined as $p < 0.05$. Analyses were performed using GraphPad Prism.

When MMB was administered immediately after 24 hours of fasting with free access to water, the blood glucose levels of Control 1 and 2 (C1, C2), which were collected before MMB administration under restraint without anesthesia, were around 100 mg/dL, and no significant changes were observed in blood glucose levels at 5, 10, and 15 minutes after MMB administration (each value correspond to T1, T2, T3, respectively). When MMB was administered immediately after 24 hours of fasting and water deprivation, there was a tendency toward hypoglycemia, but no significant changes in blood glucose levels of T1, T2 and T3). When, after 22 hours of fasting and water deprivation, a one-hour period of free feeding and drinking was provided, followed by one hour of fasting and water deprivation, and then MMB was administered immediately afterward, the blood glucose levels of C1, C2, T1, T2, T3 were 131.2 ± 3.0, 139.5 ± 6.3, 148.5 ± 6.0, 169.0 ± 5.8, 183.7 ± 8.0, respectively. While base line level of the blood glucose (C1, C2) in fasting rats was around 100 mg/dL, rats with 1 hour feeding showed higher base line level of the blood glucose. There was a significant increase in blood glucose levels of T2 and T3 after MMB administration, ($F(4, 25) = 12.88$, $p < 0.05$).

From these results, it became clear that the increase in blood glucose caused by MMB occurs prominently when the postprandial rise in blood glucose is sustained. Therefore, in subsequent experiments, a 1-hour period of free feeding and drinking was established after 22 hours of fasting and water deprivation, and then measurements were taken immediately after an additional 1 hour of fasting and water deprivation. No significant changes were observed in any of the blood glucose levels at T1, T2, or T3 under the same fasting and water

deprivation schedule, but with sham injections (no needle insertion) and intraperitoneal as well as intravenous administration of physiological saline.

We also investigated changes in blood glucose levels during the administration of propofol, an intravenous anesthetic agent commonly used for general anesthesia in current clinical practice. Intravenous administration of propofol tended to reduce blood glucose levels, but this decrease was not statistically significant. On the other hand, although this is not a standard method, when propofol was administered intraperitoneally, there were no significant changes in blood glucose levels. Additionally, unlike the rapid onset of general anesthesia following intravenous administration, rats remained awake and did not fall under general anesthesia even 15 minutes after intraperitoneal administration of propofol.

We next measured changes in blood glucose levels after administration of the inhalational anesthetics SEVO and ISO. SEVO and ISO caused a significant increase in blood glucose levels as early as 5 minutes after administration, and this significant elevation persisted at 10 and 15 minutes (ISO: $F(4, 25) = 36.90$, $p < 0.01$; SEVO: $F(4, 25) = 11.72$, $p < 0.01$). The blood glucose level before sevoflurane administration (C2) was 126.2 ± 1.6 mg/dL, and the maximum blood glucose level after administration was 151.0 ± 5.2 mg/dL. In contrast, the blood glucose level before ISO administration (C2) was 127.2 ± 2.4 mg/dL, and the maximum blood glucose level after administration was 196.7 ± 7.6 mg/dL. The increase rate in blood glucose levels with SEVO administration was 19.7%, while with ISO administration it was 54.6%. These results reveal that the blood glucose–increasing effect of ISO is approximately 2.8 times stronger than that of SEVO, demonstrating a remarkably potent effect.

Hyperglycemia induced by the triple combination anesthesia is unlikely to occur in a hypoglycemic state following fasting. However, when the blood glucose concentration is higher than the fasting blood glucose level and glucose-induced insulin secretion is ongoing, medetomidine, a component of the triple combination anesthesia, induces hyperglycemia by suppressing insulin secretion via α2 receptors. In both the propofol i.v. and propofol i.p. groups, there were no significant changes in blood glucose levels at any measurement point—5, 10, or 15 minutes after administration. These results are consistent with previous studies (Myles et al, 1995; Zuurbier et al, 2008; Kitamura et al, 2009; Tanaka et al, 2011; Yasuda et al, 2013; Sato et al, 2013) suggesting less effect of propofol on blood glucose dynamics. This may be associated with propofol's insulin secretion-promoting effect, as indicated by previous studies (Li et al, 2014; Sato et al, 2013; Sato et al, 2020). The inhalation anesthetics SEVO and ISO both showed a significant hyperglycemic effect as early as 5 minutes after administration. The results of this study indicated that the hyperglycemic effect of ISO was up to 2.8 times stronger than that of SEVO.

As described above, MMB, SEVO, and ISO were found to induce acute hyperglycemia within 5 to 10 minutes after administration. The findings obtained from this study are highly valuable for considering the effects of general anesthetics on glucose dynamics.