Original Article

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Effect of propofol on salivary secretion from the submandibular, sublingual, and labial glands during intravenous sedation

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Background: Recent animal studies have suggested the role of GABA type A (GABA-$_A$) receptors in salivation, showing that GABA-$_A$ receptor agonists inhibit salivary secretion. This study aimed to evaluate the effects of propofol (a GABA-$_A$ agonist) on salivary secretions from the submandibular, sublingual, and labial glands during intravenous sedation in healthy volunteers.
Methods: Twenty healthy male volunteers participated in the study. They received a loading dose of propofol 6 mg/kg/h for 10 min, followed by 3 mg/kg/h for 15 min. Salivary flow rates in the submandibular, sublingual, and labial glands were measured before, during, and after propofol infusion, and amylase activity was measured in the saliva from the submandibular and sublingual glands.
Results: We found that the salivary flow rates in the submandibular, sublingual, and labial glands significantly decreased during intravenous sedation with propofol ($P < 0.01$). Similarly, amylase activity in the saliva from the submandibular and sublingual glands was significantly decreased ($P < 0.01$).
Conclusion: It can be concluded that intravenous sedation with propofol decreases salivary secretion in the submandibular, sublingual, and labial glands via the GABA-$_A$ receptor. These results may be useful for dental treatment when desalivation is necessary.

Keywords: Amylases; GABA-$_A$ Receptor; Intravenous Injections; Propofol; Salivary Flow Rate; Sedation.

INTRODUCTION

Intravenous sedation is frequently used during dental and oral surgical procedures such as implant surgery [1,2]. Propofol is very useful for patients with dental phobia and gag reflex; however, it is important to restrict the saliva flow as several dental treatment procedures require the tooth cavity and pulp chamber to be dry [3-5]. Propofol, similar to other intravenous anesthetics and positive modulators of gamma-aminobutyric acid (GABA) transmission, may exert its pharmacological effects by enhancing the function of GABA-activated chloride channels [6,7]. Some animal studies have suggested that GABA type A (GABA-$_A$) receptors are involved in salivary secretion and that the inhibition of this secretion can be enhanced by GABA-$_A$ receptor agonists [8]. In addition, benzodiazepines, which bind to GABA-$_A$ receptors, produce a wide range of adverse effects such as xerostomia and hyposalivation [9,10].

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From another point of view, a decrease in salivary secretion is advantageous for dental treatment only during intravenous sedation with GABA-$_A$ receptor agonists such as propofol.

Although early studies on propofol reported increased salivation [11,12], subsequent reports [13,14] concluded that propofol reduced or caused no significant changes in salivary excretion. These findings prompted both pro- and con-arguments regarding the effects of propofol on salivary secretion. In two studies [11,12], the analysis results (data) of samples collected during induction or surgery under general anesthesia revealed hyper-salivation. Hypersalivation may be caused by stimulation resulting from a procedure or operation in which the patient is sedated. Therefore, salivary secretion must be evaluated without intervention during or after sedation with propofol. This study aimed to evaluate the effects of propofol on salivary secretion from the submandibular, sublingual, and labial glands during intravenous sedation in healthy volunteers.

METHODS

1. Participants

Twenty healthy male volunteers (age range: 22–43 years) participated in this study. They were categorized according to the American Society of Anesthesiologists physical status 1, and eight of them were smokers. The mean age was 29.9 ± 1.1 years, and the mean body weight was 74.3 ± 1.4 kg. The study was approved by the Ethics Committee of Ohu University (Approval Number 190) and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

2. Propofol infusion

An intravenous catheter (Insyte TM 22 gauge; Becton Dickinson, USA) was inserted into the median cubital vein, and lactated Ringer's solution was infused at 2 mL/kg/h. The participants were placed in a sitting position on a dental chair for 20 min. After confirming that the cardiovascular parameters were in a steady state (change in vital signs of less than 10%), the participants were sedated with propofol (a loading dose of 6 mg/kg/h for 10 min and a continuous infusion dose of 3 mg/kg/h for 15 min), as shown in Fig. 1.

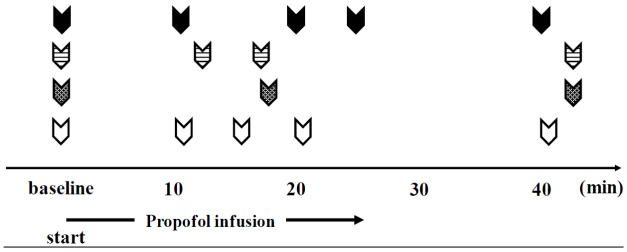

Fig. 1. Time schedule in this study. MBP, PR, and RASS were measured at 0 (baseline), 5, 10, 20, 25, and 40 min after the start of propofol infusion ( ). Salivary flow rates in the submandibular and sublingual glands were measured at 0 (baseline), 13, 18, and 43 min after the start of propofol infusion ( ). Amylase activity was measured at 0 (baseline), 18, and 43 min after the start of propofol infusion ( ). Salivary flow rates in the labial gland were measured at 0 (baseline), 11, 16, 21, and 41 min after the start of propofol infusion ( ). MBP, mean blood pressure, PR, pulse rate; RASS, Richmond Agitation Sedation Scale.

3. Measurements of mean blood pressure, pulse rate, and sedation level

The mean blood pressure (MBP) and pulse rate (PR) of the participants were measured at 0 (baseline), 5, 10, 20, 25, and 40 min after the start of propofol infusion (Fig. 1) using a biological information monitor (Colin BP-88TM; Colin, Tokyo, Japan). Similarly, the sedation level was evaluated at 0 (baseline), 5, 10, 20, 25, and 40 min after the start of propofol infusion using the Richmond Agitation Sedation Scale (RASS) [15].

4. Sampling and measurement methods

1) Salivary flow rates in the submandibular and sublingual glands

Saliva samples from the submandibular and sublingual glands were collected, and salivary flow rates were measured at 0 (baseline), 13, 18, and 43 min after the start of the propofol infusion. Cotton rolls were placed behind both lingual arches to prevent salivary inflow from the parotid glands to the sublingual space, which was

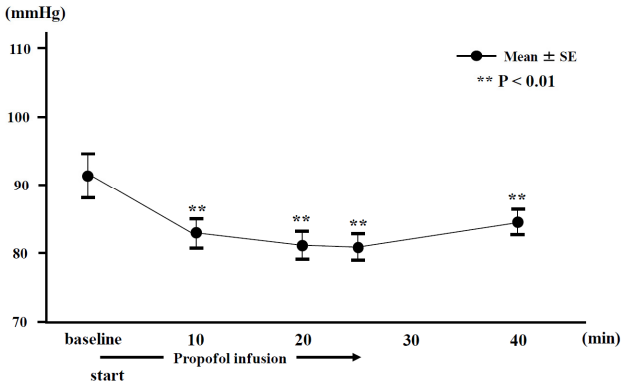

Fig. 2. Changes in the MBP. The MBP significantly decreased at 10, 20, 25 and 40 min after the administration of propofol to 83 ± 2, 82 ± 2, 81 ± 2, and 84 ± 2 mmHg, respectively ($P < 0.01$). MBP, mean blood pressure; SE, standard error.

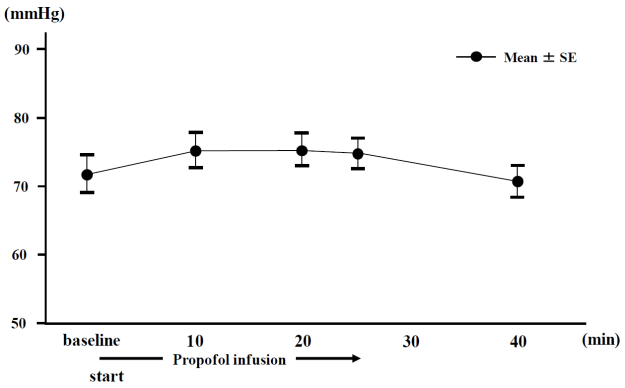

Fig. 3. Changes in the PR. The PR showed no significant differences at 10, 20, 25, and 40 min after the start of propofol infusion. The average value changed from 71 to 76 bpm. bpm, beats per minute; PR, pulse rate; SE, standard error.

soaked using additional cotton rolls before the measurements. More cotton rolls were placed in the sublingual space for 1 min to soak the residual saliva from the submandibular and labial glands. The increase in weight of the cotton roll containing saliva was measured, and the salivary flow rates in the submandibular and sublingual glands were calculated as follows: 1 g (weight) = 1 mL (volume).

2) Measuring amylase activity in the saliva from the submandibular and sublingual glands

Amylase activity was measured at 0 (baseline), 18, and 43 min after the start of the propofol infusion (Fig. 1) using a salivary amylase monitor (Nipro Co., Osaka, Japan) and a test strip (Nipro Co., Osaka, Japan) [16]. Cotton rolls were placed behind the lingual arches to prevent salivary inflow from the parotid gland into the sublingual space. Simultaneously, saliva secreted from the submandibular and sublingual glands was collected from the sublingual space for 1 min. The test strip was placed under the tongue for 30 s and amylase activity was measured using a salivary amylase monitor. The presence of amylase in the saliva secreted from the submandibular and sublingual glands was considered an indicator of amylase activity. The amylase activity was calculated for 1 min using the following formula: (measured value of salivary amylase monitor [kU/L]) × (salivary secretion volume [mL/min]).

3) Salivary flow rate in the labial gland

Saliva was sampled from the labial gland, and salivary flow rates were measured at 0 (baseline), 11, 16, 21, and 41 min after the start of the propofol infusion (Fig. 1). Salivation of the labial gland was performed using the iodine starch filter paper method [17], with minor modifications. The test paper (a 10 mm × 30 mm strip of filter paper 1; Toyo Roshi Kaisha Ltd., Tokyo, Japan) was painted with a solution of iodine in absolute alcohol, and soluble starch powder mixed with castor oil was applied to the designated location on the lower lip (inferior edge, 3 mm from the bottom of the vestibule, centered over the frenulum) for 1 min. The test areas were isolated using cotton rolls and dried with a tissue paper for 1 min before recording. The imprints made from the secreted droplets were stained black using the iodine–starch reaction. The number of black-stained spots indicated the number of actively secreting glands during the 1-min application time. Objective measurements of the sizes of the black-stained spots were performed by scanning and digitizing the area using an image scanner (DR-2510C™; Canon Co., Ltd., Japan) with a scanning resolution of 300 dpi. Each digital image was displayed on an LCD screen and converted to a black-and-white image using the free graphics editor GIMP 2.18.

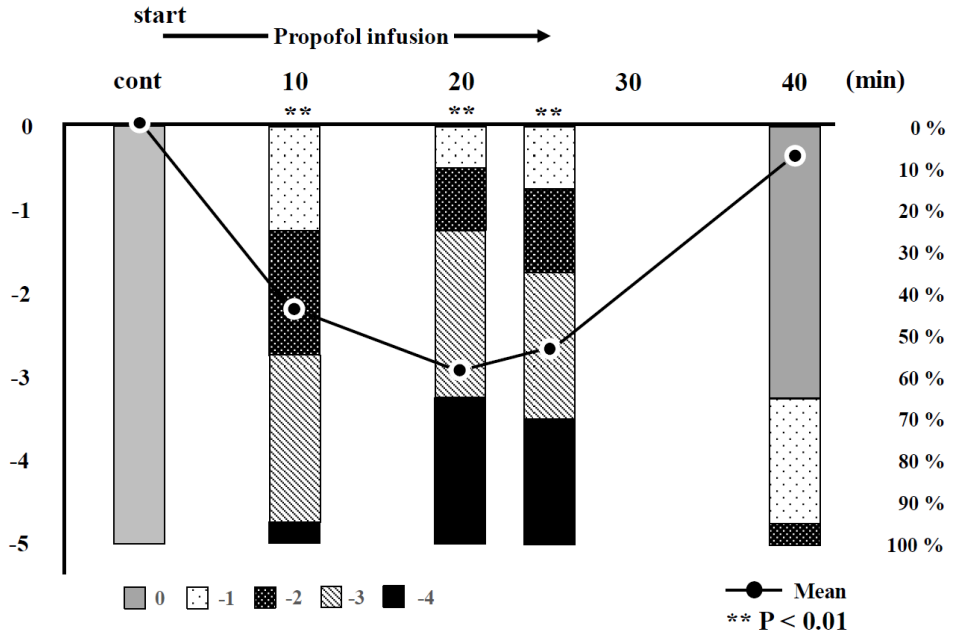

Fig. 4. Change in the RASS. Significant ($P < 0.01$) decreases in the RASS were observed at 10 (-2.3 ± 6.4), 20 (-3.0 ± 0.2), and 25 (-2.8 ± 0.2) min after the administration of propofol. However, the RASS 40 min after the start of propofol infusion showed no significant decrease. This indicates that the participants were thought to be awake from the depth of anesthesia. RASS, Richmond Agitation Sedation Scale.

Subsequently, the total area was calculated using the NIH Image software.

5. Statistical analysis

Values are expressed as the mean ± standard error of the mean. Friedman's X2r-test was employed for the statistical analysis, followed by the Wilcoxon t-test with Bonferroni's correction. Statistical significance was set at $P < 0.05$.

RESULTS

1. Participants

A total of 20 of male healthy volunteers (mean age, 29.9 ± 1.1 years; mean body weight, 74.3 ± 1.4 kg) were enrolled in this study. Each participant was sedated between 10:00 and 19:00 hours. Saliva samples were collected from the submandibular, sublingual, and labial glands, simultaneously. In our study, eight participants were smokers. The participants were not allowed to eat, drink, or smoke for 3 h before sample collection, and none of them consumed any medication or had complications.

2. MBP, PR, and RASS

The MBP significantly decreased at 10, 20, 25, and 40 min after the administration of propofol to 83 ± 2, 82 ± 2, 81 ± 2, and 84 ± 2 mmHg, respectively ($P < 0.01$) (Fig. 2). No significant differences in the PR were observed 10, 20, 25, and 40 min after the start of the propofol infusion (from an average of 71 to 76 bpm) (Fig. 3). Significant ($P < 0.01$) decreases in RASS were observed at 10 (-2.3 ± 6.4), 20 (-3.0 ± 0.2), and 25 (-2.8 ± 0.2) min after the administration of propofol (Fig. 4).

3. Salivary flow rates in the submandibular and sublingual glands

Among all participants, eighteen showed a significant decrease in salivary flow rates from the submandibular and sublingual glands during intravenous sedation with propofol. The salivary flow rates in the submandibular and sublingual glands significantly decreased ($P < 0.01$) at 13 (0.07 ± 0.01 mL/min) and 18 (0.08 ± 0.02 mL/min)

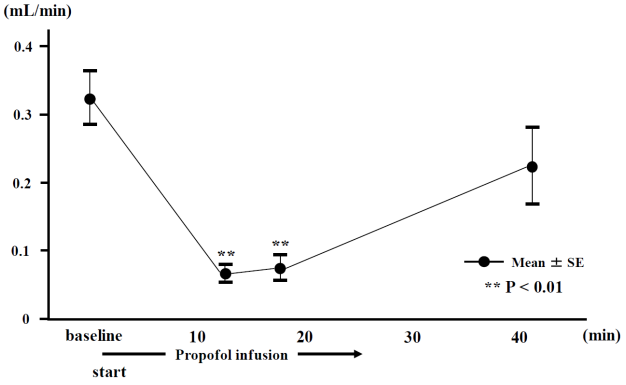

Fig. 5. Changes in the salivary flow rates in the submandibular and sublingual glands. The salivary flow rates in the submandibular and sublingual glands significantly decreased ($P < 0.01$) at 13 (0.07 ± 0.01 mL/min) and 18 (0.08 ± 0.02 mL/min) min after the administration of propofol when compared with the baseline (0.32 ± 0.04 mL/min). SE, standard error.

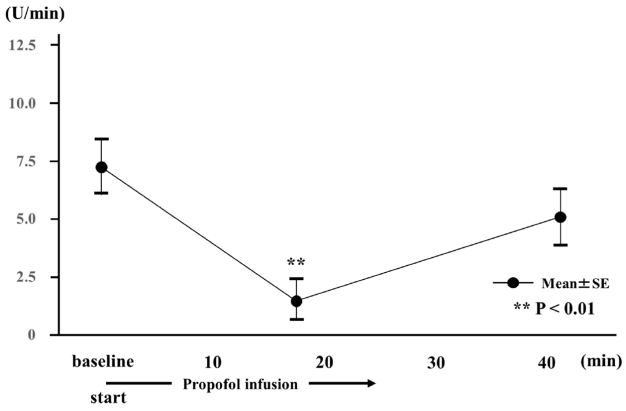

Fig. 6. Change in the amylase activity in the saliva from the submandibular and sublingual glands. Amylase activity was significantly ($P < 0.01$) decreased at 18 min (1.2 ± 0.4 U/min) after the administration of propofol when compared with the baseline (7.4 ± 1.5 U/min). However, no significant difference was observed at 43 min (5.0 ± 1.2 U/min). SE, standard error.

min after the administration of propofol when compared with the baseline (0.32 ± 0.04 mL/min) (Fig. 5).

4. Amylase activity in the saliva from the submandibular and sublingual glands

Among all participants, seventeen showed a significant decrease in amylase activity in the saliva from the submandibular and sublingual glands during intravenous sedation with propofol. The amylase activity significantly ($P < 0.01$) decreased at 18 min (1.2 ± 0.4 U/min) after the administration of propofol when compared with the baseline (7.4 ± 1.5 U/min) (Fig. 6).

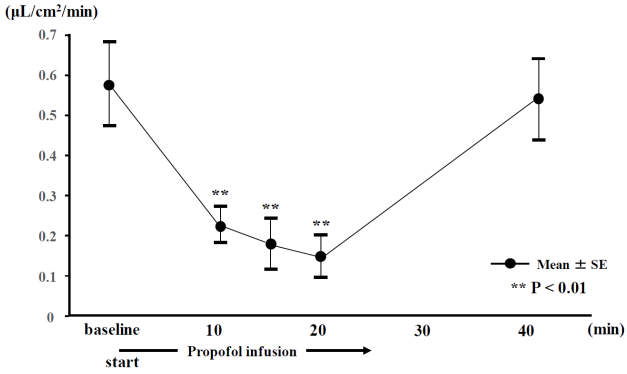

Fig. 7. Change in the salivary flow rates in the labial glands. The salivary flow rates in the labial glands at 11, 16, and 21 min after the administration of propofol were significantly ($P < 0.01$) decreased to 0.22 ± 0.04, 0.18 ± 0.06, and 0.15 ± 0.05 $\mu L/cm^2$/min, respectively, compared with the baseline of 0.57 ± 0.09 $\mu L/cm^2$/min. However, no significant difference was observed at 41 min (0.54 ± 0.10 $\mu L/cm^2$/min). SE, standard error.

5. Salivary flow rate in the labial glands

In all participants, salivary flow rates in the labial glands decreased at each point during intravenous sedation with propofol. The salivary flow rates in the labial glands at 11, 16, and 21 min after the administration of propofol were significantly ($P < 0.01$) decreased to 0.22 ± 0.04, 0.18 ± 0.06, and 0.15 ± 0.05 μL/cm^2/min, respectively, compared with the baseline of 0.57±0.09 μL/cm^2/min (Fig. 7).

DISCUSSION

Intravenous sedation is an anesthesia method used during dental treatment in patients with dental phobia or a gag reflex. However, saliva needs to be eliminated during dental treatments such as endodontic treatment and composite resin restorations. The dosage of propofol can be easily adjusted to control the depth of anesthesia; thus, propofol provides both general anesthesia and intravenous sedation [2]. Considering that propofol is frequently used in intravenous sedation for dental treatment, we evaluated how propofol controls salivary secretion during intravenous sedation with propofol.

1. Factors that can affect the participant's salivation

In general, as individuals age, their mouths become drier [18]. If the participants were compromised by people of all ages, we would encounter difficulties, such as differences in drug sensitivity and the participants' use of various medications. Moreover, elderly participants might have had prior xerostomia. These problems would cause variations in the data. This is why the age range in our study was narrow and the participants were relatively young.

From the point of view of a necessity to rule out the potential influence of diurnal variations in salivation, in our study, sedation with propofol and the collection of saliva samples were performed between 10:00 and 19:00 hours. Ferguson and Fort [19] reported the submandibular gland salivary flow rates to be constant at approximately 0.6 mL/min from 10:00 to 22:00 hours, but decrease in the morning at midnight. Hence, the saliva sample collection was not affected by the potential influence of diurnal variations in salivation.

Regarding smoking history, eight participants were smokers. One report [20] indicated no significant difference in the amount of saliva between smokers and nonsmokers. In addition, the participants in the current study were younger than those in the previous report. Therefore, we anticipated that the inclusion of smokers as participants would not pose any problems.

2. Amylase activity in the saliva from the submandibular and sublingual glands

The measurement method using a salivary amylase monitor was adopted in this study because it is easy to perform and less invasive. The test strip was placed under the tongue for 30 s. Moreover, it took only approximately 1 min to place the strip in the saliva and obtain results. In the present study, the amount of salivary secretion was thought to change at each point depending on the propofol used. Even if the amounts of amylase secreted from the submandibular and sublingual glands were the same, the concentration of amylase was higher when the amount of salivary secretion was lower. For this reason, we needed to not only measure the amount of secreted amylase but also to calculate the amount of secreted amylase per unit time. Amylase activity was calculated as follows: ([measured value of the salivary amylase monitor [kU/L]) × (salivary secretion volume [mL/min]).

In the present study, amylase activity significantly decreased at 18 min after propofol administration. This might be attributed to the sympathetic inhibition of propofol, as the RASS group exhibited a significant decrease in the depth of anesthesia from 10 min to 25 min after the administration of the agent. Sympathetic nerves are involved in protein secretion in the salivary gland, and secretion of proteins, such as salivary amylase, is regulated by the sympathetic nervous-adrenomedullary system, which is controlled by norepinephrine in the salivary glands [21,22].

In addition, the importance of sympathetically mediated impulses in evoking protein secretion has been demonstrated in a previous study in which electrical stimulation of sympathetic nerves stimulated the secretion of proteins in the rat parotid [23]. However, the MBP decreased slightly during sedation with propofol, and no significant difference in the PR was observed in the current study. From the perspective of hemodynamic changes, it is unlikely that propofol inhibits sympathetic nerve activity. Conversely, Okubo and Kawaguchi [24] reported that benzodiazepines inhibited beta-adrenoceptor- and muscarinic receptor-stimulated amylase release. Therefore, in the present study, inhibition of protein secretion from the salivary glands during propofol infusion may be attributed to an underlying mechanism unrelated to sympathetic nerve activity.

3. Salivary flow rates in the submandibular, sublingual, and labial glands

Salivary flow rates in the submandibular and sublingual glands significantly decreased at 13 and 18 min, and those in the labial glands significantly decreased at 11, 16, and 21 min after propofol administration. These decreases were observed at each time point during propofol

infusion. The decrease in sympathetic activity caused by propofol is partially due to its inhibitory effect on hypothalamic paraventricular nucleus neurons [25]. Furthermore, propofol reduces sympathetic tone to a greater extent than parasympathetic tone, resulting in a predominance of parasympathetic responses [26]. Neurologically, salivary secretion is controlled by sympathetic and parasympathetic nerves in the salivary glands [27]. Parasympathetic impulses usually evoke more fluid secretion into the saliva than sympathetic nerves [28]. In addition, minor glands secrete approximately 5% of the total volume of the relative contribution of each type of gland to the total volume secreted, and the labial glands are thought to lack sympathetic secretory innervation [29]. From the aspect of autonomic nervous activity, our results of salivary flow rate in the submandibular, sublingual, and labial glands are irreconcilable with the neurological theory. Thus, it is unlikely that salivary flow rates in the submandibular, sublingual, and labial glands decreased because of the propofol infusion. The rates in these glands may have been decreased by a working mechanism unrelated to autonomic nervous activity.

4. Blood flow in the salivary glands

As a factor to influence to the salivary flow rates except for autonomic nerves, blood flow in the salivary glands is thought to be one of the underlying mechanisms to decrease the salivary flow rates during propofol infusion. Because the salivary fluid is a mixture of water and ions produced from blood plasma in each gland [27], in other words, the salivary flow rates are thought to depend on the blood flow in each salivary gland. Neurologically, the submandibular and sublingual glands are controlled by both sympathetic and parasympathetic nerve activities. Sato and Ishii [30] reported that blood flow in these two glands and the sublingual gland was increased by parasympathetic nerve activity; excitement of the cholinergic nerves increased blood flow in the submandibular glands, whereas cholinergic and non-cholinergic nerve excitement increased blood flow in the sublingual glands. Izumi et al. [31] reported that electrical stimulation of the superior cervical sympathetic trunk reduced the blood flow increase-mediated parasympathetic nerve activity in the lower lip. Therefore, blood flow in the submandibular, sublingual, and labial glands is thought to increase or remain unaltered due to the inhibitory effect of propofol on sympathetic nerve activity. However, the results of the current study demonstrated a decrease in salivary flow rates in the three types of glands, indicating that the salivary flow rates in these glands were not influenced by the inhibitory effect of propofol on the sympathetic nerve activity. Conversely, Ichinohe et al. [32] reported that propofol did not change the mucosal blood flow in rabbits. Furthermore, Nakamura et al. [33] demonstrated that clinically relevant concentrations of propofol did not have a direct vasodilating effect. Therefore, the decrease in the salivary flow rate might not be attributed to the vasodilating effect of propofol, as propofol might not have any effect on blood flow in the submandibular, sublingual, and labial glands.

5. Effect of the GABA receptor

GABA is widely distributed in the mammalian central nervous system, where it acts as a major mediator of synaptic inhibition. The presence of GABA and its biosynthetic and metabolic enzymes in salivary glands has been demonstrated in animal experiments [34]. Okubo and Kawaguchi [35] demonstrated a dose-dependent decrease in salivary secretion after perfusion with a GABA agonist (benzodiazepine), which was recovered using a GABA-$_A$ receptor antagonist. Further, as an action mechanism of the decrease in salivary secretion induced by a GABA agonist (benzodiazepine), Kujirai et al. [36] reported that diazepam (DZP) inhibited muscarinic receptor-stimulated inositol 1,4,5-trisphosphate (IP3) production through benzodiazepine receptors and that DZP attenuated the increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) following stimulation of the muscarinic and α 1-adrenoreceptors. Consequently, their results suggest

that the suppression of salivary secretion induced by DZP may be related to a decrease in both IP3 and $[Ca^{2+}]_i$ in the cells. Kosuge et al. [37] reported that benzodiazepines such as clonazepam decrease salivary secretion mediated by the GABA-$_A$ receptor. Clonazepam has an effective binding site for benzodiazepines on the GABA-$_A$ receptor and forms a complex with chloride channels. Propofol, similar to other intravenous anesthetics and positive modulators of GABA-ergic transmission, may exert its pharmacological effects by enhancing the function of GABA-activated chloride channels [6,7]. In addition, propofol potentiates the positive modulation of the inhibitory action of GABA through GABA-$_A$ receptors [8], indicating that it has the same effect as clonazepam. Therefore, the decrease in salivary flow rates observed in the present study might be associated with the propofol binding site on the GABA-$_A$ receptor.

In conclusion, the findings of this study suggest that intravenous sedation with propofol decreases salivary secretion in the submandibular, sublingual, and labial glands via GABA-$_A$ receptors. Thus, intravenous sedation with propofol may prove useful for dental treatment, particularly in cases where desalivation is necessary.

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AUTHOR CONTRIBUTIONS

Keisuke Masuda: Investigation, Writing - original draft
Akira Furuyama: Data curation, Formal analysis, Investigation, Methodology, Project administration
Kenji Ohsuga: Data curation, Investigation, Resources
Shota Abe: Data curation, Investigation, Methodology
Hiroyoshi Kawaai: Formal analysis, Investigation, Writing - review & editing

DECLARATION OF INTERESTS: The authors of this paper have no conflicts of interest to declare.

NOTE: We have registered the number of Clinical Trial Registration for our clinical research.
CLINICAL TRIAL NUMBER: UMIN 000051146.

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