Initial Transient Phase of Salivary Secretion by the Submandibular Glands of Rats under Anoxic Conditions

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Abstract: Salivary secretion of the rat submandibular gland exhibits two phases upon administration of acetylcholine (ACh, $10^{-6}$M): an immediate initial transient phase of rapid secretion lasting 5 min followed by a longer steady phase of slower secretion. Application of an anoxic perfusate bubbled with 100% N$_2$ for 10–20 min had no effect on the initial phase of secretion, but caused a marked decrease in secretion in the steady phase following stimulation with $10^{-6}$M ACh. After secretion under the anoxic condition, a recovery period without stimulation was performed for 30 min by perfusion with HEPES Ringer's solution bubbled with 100% O$_2$ and containing various potassium concentrations. The initial secretion rate measured with application of an anoxic perfusate was markedly increased by the high-K$^+$ recovery perfusate (25 mM) and decreased by the K$^+$-free recovery perfusate. Administration of $10^{-3}$M ouabain in the normal perfusate resulted in inhibition of secretion during the steady phase similar to that seen under the anoxic condition, while the secretory rate during the initial phase remained unchanged. We concluded that the initial phase of secretion is relatively resistant to anoxia, and that oxygen supply and Na$^+$-K$^+$ pump activity were essential for maintaining the steady phase of secretion. [Japanese Journal of Physiology, 46, 155–161, 1996]

Key words: submandibular gland, salivary secretion, gravimetric method, electrochemical potential.

Since the development of submandibular gland perfusion techniques allowed immediate changing of the perfusate, many investigators have studied the ion transport mechanism of the submandibular gland and the effect of oxygen on salivary secretion [1–6]. Imai [2] reported that the rate of secretion of the dog submandibular glands after high-potassium perfusion increased markedly upon stimulation with ACh in the normal perfusate. Murakami et al. [7] reported that the rate of K$^+$ uptake increased during stimulation as well as post-stimulation. Thus, the rate of salivary secretion seems to be correlated with the potassium content in the gland.

Salivary secretion exhibits two phases, an initial phase and a steady phase during administration of ACh [1, 4, 8, 9]. We examined the effect of potassium on the rate of secretion in the initial phase, especially under anoxic conditions of perfusion. The results suggest that the electrochemical potential gradient of ions across the cell membrane played an important role on the secretory mechanism in the initial phase of secretion.

MATERIALS AND METHODS

Adult female albino rats of the Wistar/ST strain, weighing 250–300 g, were anesthetized with intraperitoneal injections of pentobarbital sodium (Nembutal, Dainabot Co.) at 25 mg/kg body weight. The submandibular glands were dissected under a microscope. Details of the procedure for the isolation and perfusion of this gland were the same as those previously described [10, 11]. The glands were perfused arterially at a rate of 2 ml/min using a peristaltic pump (Cole Palmer Instrument Co.) and stimulated with $10^{-6}$M ACh. HEPES Ringer's solution (normal per...
Fig. 1. Schematic illustration of the experiments. Experiments were performed under various conditions as follows. 1) Gas contents: (i) 100% O₂, (ii) 0% O₂, 100% N₂. 2) Ouabain administration. 3) Various K⁺ concentrations: (i) 4.3 mM K⁺, (ii) 0 mM K⁺, (iii) 25 mM K⁺. The perfusion rate was regulated with a peristaltic pump (2 ml/min). A polyethylene cannula was inserted into the main duct of the salivary gland and into the feeding artery. Secreted saliva was collected in the sampling cup on the electric balance. A computer was used for collection of data and the calculation of flow rate.

RESULTS

1. Stimulation under normal oxygenated and anoxic conditions

The initial transient phase of high secretory rate occurred immediately after stimulation with 10⁻⁶ M ACh and lasted 5 min, and then the prolonged steady phase of low secretory rate occurred (Fig. 2A). The peak secretory rate in the initial phase under the oxygenated condition was around 219 μl·min⁻¹·g⁻¹ (Table 1). The secretory rate in the steady phase beginning 10 min after stimulation was around 98 μl·min⁻¹·g⁻¹ (Table 1).

Under the anoxic condition, the initial phase of secretion stimulated with 10⁻⁶ M ACh occurred as it did under the oxygenated condition; however, the steady phase of secretion did not occur (Fig. 2B). The peak secretory rate in the initial phase under the anoxic condition was around 100 μl·min⁻¹·g⁻¹ (Table 1). The initial transient phase and the steady phase were not observed upon re-stimulation during the anoxic condition (Fig. 3A). However, the steady phase response recovered after application of oxygenated perfusate for around 15 min (Fig. 2B).

The initial phase of secretion occurred upon re-
stimulation under the anoxic condition when the oxygenated normal Ringer's solution containing 4.3 mM K$^+$ was applied as the recovery perfusate for 30 min after the first stimulation under the anoxic condition. The pattern of the second secretion was the same as that of the first (Fig. 3B).

2. Effect of various K$^+$ concentrations in the recovery perfusate on the initial phase of secretion under the anoxic condition

The peak secretory rate in the initial phase under the anoxic condition upon re-stimulation became around 127 μl·min$^{-1}$·g$^{-1}$ when 25 mM K$^+$ concentration Ringer's solution was applied as the oxygenated recovery perfusate for 30 min (Fig. 4A and Table 1).

The peak secretory rate in the initial phase under the anoxic condition upon re-stimulation became around 21 μl·min$^{-1}$·g$^{-1}$ when 0 mM K$^+$ Ringer's solution was applied as the oxygenated recovery perfusate for 30 min (Fig. 4B and Table 1).

We investigated the effect of administration of ouabain (10$^{-3}$ M) in the normal Ringer's solution for 30 min under the oxygenated condition after the first stimulation. Then we stimulated the preparation with ACh for the second time under the oxygenated condition. We found that ouabain decreased the secretory

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**Table 1. Peak and mean secretory rates in the initial transient phase.**

<table>
<thead>
<tr>
<th></th>
<th>Peak secretory rate (initial phase) (μl·min$^{-1}$·g$^{-1}$)</th>
<th>Mean secretory rate for initial 5 min (μl·min$^{-1}$·g$^{-1}$)</th>
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</thead>
<tbody>
<tr>
<td>K$^+$: 4.3 mM</td>
<td>99.8±10.8 (n=11)</td>
<td>35.4±4.46 (n=11)</td>
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<tr>
<td>(100% N$_2$)</td>
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<tr>
<td>K$^+$: 4.3 mM</td>
<td>218.5±19.2 (n=11)</td>
<td>80.3±14.9 (n=11)</td>
</tr>
<tr>
<td>(100% O$_2$)</td>
<td></td>
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<tr>
<td>K$^+$: 25 mM</td>
<td>127.4±28.5 (n=10)</td>
<td>42.3±7.3 (n=10)</td>
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<tr>
<td>(n=5)</td>
<td></td>
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</tr>
<tr>
<td>K$^+$: 0 mM</td>
<td>20.9±1.3 (n=5)</td>
<td>9.0±2.7 (n=5)</td>
</tr>
<tr>
<td>(100% N$_2$)</td>
<td></td>
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<tr>
<td>Ouabain</td>
<td>62.7±20.3 (n=5)</td>
<td>25.3±5.6 (n=5)</td>
</tr>
<tr>
<td>(100% O$_2$)</td>
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</tbody>
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rate in the initial phase, and suppressed the steady phase. The peak secretory rate in the initial phase following ouabain administration was around 63 μl·min⁻¹·g⁻¹ (Fig. 2A and Table 1).

Table 1 summarizes the peak secretory rate and the mean secretory rate in the initial phase. The highest peak secretory rate in the initial phase was obtained under the oxygenated condition. However, we assumed that this value contained both the initial secretion and the continuous secretion (Fig. 2A and Table 1). Therefore, we assumed that the true peak secretory rate in the initial phase could be calculated by deducting the continuous secretion from the apparent initial secretion; these values are shown in the table in parentheses, and should be compared with the peak secretory rates in the initial phase under the anoxic condition (Fig. 3B and Table 1). The true peak secretory rate in the initial phase under the oxygenated condition and that in the initial phase under the anoxic condition were not significantly different (p<0.01). The peak secretory rate in the initial phase upon re-stimulation under the anoxic condition was increased by application of the high-K⁺ recovery perfusate, and was decreased by application of the low-K⁺ recovery perfusate. The peak secretory rate in the initial phase in the control experiment was different from that in the initial phase in the experiment with 0 or 25 mM K⁺ in the recovery perfusate, and from that with ouabain administration (p<0.05).

The mean secretory rates were also calculated from the cumulative secretory volumes measured using an electric balance during initial 5 min. These rates in the initial phase in the control experiments were also significantly higher than those in the experiments with 0 mM K⁺ in the recovery perfusate (p<0.05).

**DISCUSSION**

Salivary secretion exhibited two distinct phases upon ACh administration under the oxygenated condition: an initial transient phase of rapid secretion and a prolonged steady phase of slower secretion. Under the
anoxic condition, however, the initial transient phase of secretion occurred, and the steady phase of secretion diminished completely.

We concluded that there were differences in the mechanism of secretion between the initial phase and the steady phase, because the initial phase of secretion was resistant to anoxia for 10–20 min, but the steady phase of secretion required sufficient oxygen supply.

Upon administration of ACh under the anoxic condition with 100% N₂ supply, the rate of secretion in the initial phase decreased slightly compared to that in the oxygenic experiment for the first stimulation. However, upon re-stimulation under the anoxic perfusate after 30 min of application of the anoxic perfusate both the initial phase and the steady phase of secretion were suppressed completely. We concluded that the driving force of the initial secretion was limited under the anoxic condition and was exhausted by repeated stimulation. In the anoxic experiments, we used 5–10 min as the anoxic preincubation time before stimulation with regard to tissue damage due to prolonged anoxia. Under the condition of anoxic state within 10 min, measurements of the initial secretion were stable (Fig. 3B) and we supposed the driving force of the initial secretion was also maintained. Figure 2 showed that both the initial and continuous secretion started simultaneously upon stimulation. True initial secretion must be calculated by deducting the continuous secretion from the apparent initial secretion. This true initial secretion was the same as the initial secretion under the anoxic condition.

Murakami et al. [6, 7] reported that K⁺ was released from the cell upon stimulation with ACh, and that K⁺ was taken into the cell immediately upon application of well-oxygenated normal Ringer’s solution after the stimulation. These observations indicated ac-
tive transport of $K^+$ against the electrochemical gradient via Na-K pump and/or Na-K-Cl symporter played an important role in $K^+$ uptake across the cell membrane. We assumed that the electrochemical potential gradient of $Na^+$ and $K^+$ across the cell membrane was very important for the occurrence of the initial phase of secretion under the anoxic condition.

Figure 2A showed that following administration of ouabain in the perfusate for 30 min, salivary secretion stimulated by ACh administration decreased in both the initial phase (slightly) and the steady phase (markedly). These observations indicated that the steady phase of secretion is much more dependent on oxygen supply, but the initial phase had another energy source for secretion. We assumed that the ion gradient across the cell membrane was formed incompletely under this condition; therefore, the initial phase of secretion was also affected by ouabain administration, leading to a decreased secretory rate. The fact that the rate of secretion in the initial phase upon re-stimulation with ACh decreased slightly after ouabain administration was attributed to Na-K pump activity in the resting and recovery phases of the acinar cells. It is well known that Na-K pump activity is required for maintenance of the high $K^+$ and low $Na^+$ environment in cells [6, 7]. We supposed that the electrochemical potential gradient across the cell membrane decreased upon application of the $K^+$-free recovering perfusion of the cell, and increased in the high $K^+$ recovery perfusate with oxygen supply. The results in Fig. 4A and B support our speculation. We further concluded that the presence of the electrochemical potential gradient across the cell membrane was the most important factor of the secretory mechanism in the initial phase.

The role of chloride on secretory mechanisms has become very important in recent years [12]. The electrochemical potential gradients of Na, K, and Cl are calculated as driving forces for the initial secretion. Assuming an intracellular : extracellular concentration of Na, K, and Cl of 10:140, 140:40, and 40:120 mm as well as intracellular electrical potential of $-50\, mV$, the driving force across the basolateral membrane of acinar cells can be estimated as $116\, mV$ for Na, $-40\, mV$ for K, and $-23\, mV$ for Cl. Since the driving force of Cl across the luminal membrane is small, if secreted saliva has the same ion composition as those in the extracellular fluid, it was impossible for intracellular chloride content to drive the whole secretory volume of the initial phase. Decreased intracellular chloride on stimulation must be supplied by some transport mechanism such as Na-K-Cl symporter, or H-Na antiporter, or Cl-HCO$_3^-$ antiporter. Therefore Na gradient across the basolateral membrane becomes the most important factor. The potential difference of K across the membrane reflects the mirror image of Na potential difference, because the total amount of Na and K concentration is maintained constant. The above-mentioned Na-K coupling is very important to maintain the driving force of the initial secretion. The dependence of the initial secretory rate on $K^+$ concentration of the recovery perfusate suggests that the electrochemical potential gradient of $K^+$ is more important than that of Cl. Further studies of intracellular Ca$^{2+}$ dynamics, cell energetics and channel involvement are required to precisely assess the mechanism of the initial secretory response.

REFERENCES

9. Ricardo Martinez J and Cassity N: Effect of ouabain, furosemide and ethacrynic acid on fluid and electrolyte secretion by the perfused rat submandibular gland. In:
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