A Method to Estimate the Transient Interstitial Osmolarity Change upon Application of Osmotic Stress in Perfused Rat Submandibular Gland

Hideyo YOSHIDA, Takashi NAKAHARI, and Yusuke IMAI

Department of Physiology, Osaka Medical College, Takatsuki, 569 Japan

Summary: Transient osmolarity changes in the interstitium of perfused rat submandibular glands were estimated by measurement of the interstitial Cl^- concentration ($[\text{Cl}^-]_{\text{ECF}}$) with an Ag-AgCl electrode upon application of osmotic stress by the addition or subtraction of NaCl. A simple model was used to describe the interstitial osmolarity changes upon the application of osmotic

stress. The measured $[\text{Cl}^-]_{\text{ECF}}$ values and interstitial osmolarities were fit well by an exponential function derived from the model. The good fit of the theoretical curve indicates that this model describes adequately the changes in interstitial osmolarity after switching the perfusate osmolarity to a new value. [Japanese Journal of Physiology, 47, 311–316, 1997]

Key words: ECF, Cl^- electrode, perfusion.

Perfusion studies are a useful means of investigating organ functions [1]. Switching of the perfusate osmolarity allows the osmotic driving force for fluid secretion to change and enables calculation of the biophysical parameters of the entire gland, such as osmotic water permeability or osmotic driving force for fluid secretion. However, many studies on perfused glands involving changes in perfusate osmolarity were performed in a plateau phase not in a transient phase [2, 3], because it is difficult to estimate interstitial fluid osmolarity following a step change of perfusate osmolarity in a vascularly perfused submandibular gland. In recent experiments, we have demonstrated that a reduction in the perfusate osmolarity causes the secretory rate to increase transiently, whereas an increase in the perfusate osmolarity causes it to decrease transiently [4, 5]. In these studies, we estimated the osmotic water permeability of the entire gland and the osmotic driving force for fluid secretion from the transient changes in fluid secretion rate and interstitial osmolarity changes [5]. In this report, we introduce a method to estimate the transient interstitial osmolarity changes in a perfused submandibular gland by switching the perfusate osmolarity to a new value.

We developed a simple model consisting of three compartments (artery, interstitium and vein) and used it to describe the changes in interstitial osmolarity ($[\text{OSM}]_{\text{ECF}}$) in perfused submandibular glands. To achieve experimental conditions which could be simulated using this simple model, we used unstimulated submandibular glands perfused at a constant rate because, in these glands, fluid movement could be assumed to occur only at the capillary wall and not between the interstitium and lumen. Furthermore, to analyze transient changes such as 5 s after switching, interstitial osmolarities have to be measured continuously. The interstitial Cl^- concentration ($[\text{Cl}^-]_{\text{ECF}}$) was measured continuously using a Cl^- -sensitive electrode (Ag-AgCl electrode) in a previous report, and it was suggested that $[\text{Cl}^-]_{\text{ECF}}$ may be a good indicator of $[\text{OSM}]_{\text{ECF}}$. In this report, we measured the $[\text{Cl}^-]_{\text{ECF}}$ using a Cl^- -sensitive electrode (Ag-AgCl electrode) after switching the perfusate NaCl concentration [3], and compared the $[\text{Cl}^-]_{\text{ECF}}$ and interstitial osmolarities measured with a theoretical curve derived from the model.

In the submandibular gland, the interstitial space is divided into the interlobular and intralobular intersti-

Received on February 6, 1997; accepted on April 14, 1997

Correspondence should be addressed to: Hideyo Yoshida, Department of Physiology, Osaka Medical College, 2-7 Daigaku-cho, Takatsuki, 569 Japan. Tel: +81-726-83-1221 ext. 2654, Fax: +81-726-84-6520, E-mail: takan@art.osaka-med.ac.jp

tial spaces [6, 7]. The fluid exchange between the two interstitial spaces is thought to be sufficiently rapid because there are no barriers between them. Fluid which enters from the arterial side of the capillaries flows through both interstitial spaces to the venous side of the capillaries. In this report, we measured the osmolarities of interlobular space as that of interstitial space.

Materials and methods

Solutions. In NaCl experiments, the control perfusate (solution A) contained (mM): Na^+ , 121; K^+ , 4.5; Ca^{2+} , 1.5; Mg^{2+} , 1; Cl^- , 100.5; HCO_3^- , 25; glucose, 5; and HEPES, 10, and had a pH of 7.4. The osmolarities of the test solutions were increased by the addition of 50 mM NaCl (solution B), 100 mM NaCl (solution C) or 150 mM NaCl (solution D) to the control perfusate (solution A), and the Cl^- concentrations of solutions A–D were 100.5, 150.5, 200.5 and 250.5 mM, respectively. The osmolarities of solutions A–D were 235, 323, 418 and 506 mosM, respectively. In sucrose experiments, the control perfusate (solution E) contained (mM): Na^+ , 151; K^+ , 4.5; Ca^{2+} , 1.5; Mg^{2+} , 1; Cl^- , 130.5; HCO_3^- , 25; glucose, 5; and HEPES, 10, and had a pH of 7.4. In a test solution (solution F), sucrose (300 mM) was added into solution E (control solution). The osmolarities of solutions E and F were 301 and 615 mosM, respectively. The osmolarities of the perfusates were measured using a freeze-point depression osmometer (model ONE-TEN, Fiske Associates, Norwood, MA, USA). The solutions were aerated with a gas mixture (95% O_2 , 5% CO_2), and the experiments were performed at 37°C.

Submandibular gland preparation and $[\text{Cl}^-]_{\text{ECF}}$ measurement. Adult male Wistar rats (slc: Wistar/ST) weighing 400–450 g were purchased from SLC Japan Inc. (Hamamatsu, Japan) and fed a standard pellet diet. The rats were anesthetized by intraperitoneal injection of pentobarbital sodium (Nembutal 60–70 mg/kg). The submandibular gland was isolated for perfusion as described elsewhere [8, 9]. The experimental setup is shown in Fig. 1A. The artery of the submandibular gland was cannulated with a polyethylene cannula and perfused using a peristaltic pump (model 7553-70; Cole-Palmer Instrument Co., Niles, IL, USA). The vein of the submandibular gland was cut. An insulated silver wire (0.2 mm in diameter), of which the exposed tip (1–2 mm) was coated with AgCl, was placed in the interlobular space of the perfused gland and fixed by strings (Fig. 1A). The reference electrode (Ag-AgCl electrode in 300 mM NaCl) was connected to the gland via a 300 mM NaCl agar bridge, which reduced the noise level of the Cl^- potential as compared to a 150 mM NaCl agar bridge. The Cl^- potential of the interlobular space was recorded by a pen-recorder (model R62A, Rikadenki Electronics Ltd., Tokyo, Japan) via a differential amplifier. Data were also stored in transient memory (SD2120, Graphtec Co., Osaka, Japan) and further analysis, such as curve fitting, was performed using a computer. An Ag-AgCl electrode, which is well-known to be Cl^- -sensitive and widely used for monitoring Cl^- concentrations [3], was calibrated using the perfusates

Fig. 1. (A) Schematic diagram of the experimental setup. (B) A model of the perfused submandibular gland. F_v is the perfusion rate. J is the exchange volume flow and P is the permeability of osmolyte. V_{ECF} is the interstitial fluid volume. c_2 and $c(t)$ are osmolyte concentration of perfusate and interstitial space at time t , respectively. Osmolytes entering the interstitial fluid are distributed immediately

throughout the interstitial space. Therefore, the amount of osmolyte entering the interstitium by convective flow is expressed as $J \cdot (c_2 - c(t))$ and that by diffusion as $P \cdot (c_2 - c(t))$. The rate of osmolyte entering the interstitium is $f \cdot (c_2 - c(t))$, where f is $(J_v + P)$. Here we assume $f = \alpha \cdot F_v$ (α is constant).

(solutions A–D) and NaCl solutions (100, 120, 150, 200, 250 and 300 mM) before and after the experiments using the same experimental setup. In the calibration, the responses of the electrode were completed within 5 s. The effect of perfusate switching on Cl^- potential was negligible. In the unstimulated submandibular glands, the effects of secretory potential on the chloride potentials were negligible. The changes in perfusate composition infused from the artery can be 95% complete after 25 and 15 s at pump rates of 2.4 and 4.3 ml/min, respectively, and are independent of the length of the tube in the perfusion system (Fig. 1A).

We also measured the osmolarity of interstitial fluid directly, which was sampled from the interlobular space by a pipet (10 μl). The sampling intervals were 30–60 s. The perfusate osmolarities were changed by the addition of NaCl (200 mM) or sucrose (300 mM).

At the end of each experiment, the submandibular gland and an adherent sublingual gland were removed from the experimental setup and weighed immediately. Then, it was blotted firmly for removal of the edema fluid and reweighed. In our previous experiments, the gland weights before and after blotting were 0.747 ± 0.076 g and 0.297 ± 0.030 g, respectively [5]. The interstitial fluid volume was 60% of the perfused gland.

Model for calculation of interstitial osmolarities following switching of the perfusate osmolarities to a new value. To estimate the changes in $[\text{OSM}]_{\text{ECF}}$, we initially made the following assumptions: (1) Fluid exchange at the capillaries was sufficiently rapid; and (2) osmolytes entering the interstitial fluid mixed instantaneously throughout the interstitial space.

A schematic diagram of the model is shown in Fig. 1B. In the perfused submandibular gland, the net volume flow across capillary walls, J_{net} , is expressed as

$$J_{\text{net}} = J_{\text{in}} - J_{\text{out}} = 0,$$

where subscripts in and out indicate influx and outflux across the capillary walls. Therefore, convective flow of an osmolyte is expressed as $J \cdot (c_2 - c(t))$, where $J (=J_{\text{in}}=J_{\text{out}})$ is the exchange volume flow. The diffusional flow of an osmolyte is expressed as $P \cdot (c_2 - c(t))$, where P is the diffusional permeability of an osmolyte at the capillaries.

The perfusate osmolyte concentration is switched suddenly from c_1 to c_2 . At time t , $[\text{OSM}]_{\text{ECF}}$ is $c(t)$ and $c(0)$ is c_1 . At time t , the influx of osmolytes is expressed by the following equation,

$$V_{\text{ECF}} \cdot dc(t)/dt = (J+P) \cdot (c_2 - c(t)),$$

where V_{ECF} is the interstitial fluid volume. Here, we put a factor, f , to $(J+P)$. Therefore, the change in $[\text{OSM}]_{\text{ECF}}$ is expressed as

$$V_{\text{ECF}} \cdot dc(t)/dt = f \cdot (c_2 - c(t)), \quad (1)$$

We assume that factor f is a function of the perfusion rate, F_v :

$$f = \alpha \cdot F_v,$$

where α lies between 0 (no exchange) and 1 (rapid exchange). When V_{ECF} and f are assumed to be constant and osmolyte exchange is assumed to occur only at the capillary walls, we obtain

$$c(t) = c_2 + (c_1 - c_2) \cdot \exp(-t/\tau), \quad (2)$$

where τ is the time constant of exchange. In the ideal case, where the interstitial fluid is well mixed and fluid exchange across the capillary walls is rapid,

$$\tau = V_{\text{ECF}}/f (=V_{\text{ECF}}/(\alpha \cdot F_v)). \quad (3)$$

Here, we used $[\text{Cl}^-]_{\text{ECF}}$ as an indicator of $[\text{OSM}]_{\text{ECF}}$. The change in the Cl^- potential ($\Delta\psi$) at time t measured using the chloride electrode is expected to be

$$\Delta\psi = \psi(t) - \psi(0) = \beta \cdot R \cdot T/F \cdot \ln(c(t)/c_1), \quad (4)$$

where β is the sensitivity of the chloride electrode, T is the absolute temperature and F and R are the Faraday and gas constants respectively. The measured data were therefore fitted using Eqs. 2 and 4 by the least-squares method, and the value of τ was determined. The values of α , which was calculated by Eq. 3 are almost constant and independent of pump rate in the present experiments. The average value of α obtained from 21 experiments was 0.22 ± 0.02 .

In the present experiments, the weight differences of the gland before and after blotting and the pump rate were used as the values for interstitial fluid volume, V_{ECF} , and perfusion rate, F_v , respectively.

Results and discussion

A record of the chloride potential, ψ , following switching of the perfusate Cl^- concentration is shown in Fig. 2. When the perfusate was switched suddenly from solution A to solution B (+50 mM NaCl) or solution C (+100 mM NaCl), ψ increased and reached a new steady-state value; the increase in ψ was about 8 mV upon switching to solution B and about 15 mV upon switching to solution C. On returning to solution A, ψ returned to the previous level. The changes in ψ were completed within 4 min. The calibrated Cl^- concentrations are also shown in Fig. 2.

Fig. 2. A record of the Cl^- potential following a step change in the perfusate Cl^- concentration in a perfused submandibular gland. The pump rate was 2.4 ml/min. The calibrated Cl^- concentrations are also shown in the figure. Upon switching of the perfusate from solution A to solution B or C, the Cl^- potential increased and reached a plateau value within 3 min. On returning to solution A, the Cl^- potential recovered to the previous level.

Fig. 3. Measured Cl^- potential change ($\Delta\Psi$) and the results of curve fitting. The filled circles represent measured $\Delta\Psi$ and the solid lines show the results fitted by Eqs. 2 and 4. **(A)** The perfusate Cl^- concentration was changed from 100.5 mM (solution A) to 200.5 mM (solution B). The pump rate was 2.4 ml/min. The value of τ obtained by curve fitting was 54.6 s, and V_{ECF} measured was 0.46 g (interstitial fluid volume of 61.3%). The calculated value of α was 0.213. **(B)** The perfusate Cl^- concentration was changed from 100.5 mM (solution A) to 150.5 mM (solution C). The pump rate was 4.3 ml/min. The value of τ obtained by curve fitting was 33.5 s, and V_{ECF} measured was 0.53 g (interstitial fluid volume of 60.5%). The calculated value of α was 0.221.

Figure 3A and B, obtained from different glands, show $\Delta\Psi$ as a function of time at two perfusion rates. The perfusion rates for Fig. 3A and B were 2.4 and 4.3 ml/min respectively. The filled circles represent the raw data ($\Delta\Psi$) collected using an Ag-AgCl electrode. The submandibular gland was first perfused with solution A, and then the perfusate was suddenly switched to solution C. After $\Delta\Psi$ had reached a new steady-state value, the perfusate was switched back to solution A and $\Delta\Psi$ returned to its initial value. The solid lines in Fig. 3 show the results fitted by Eqs. 2 and 4. The values of τ and V_{ECF} were 54.6 s and 0.46 g (the interstitial fluid volume was 61.6% of the entire perfused gland) in Fig. 3A, and 33.5 s and 0.53 g (60.1% of the perfused gland) in Fig. 3B. The values of α calculated from τ , V_{ECF} and F_v were 0.213 (Fig. 3A) and 0.221 (Fig. 3B). A good fit was also obtained on switching of the perfusate from solution A to solutions B or D (data not shown).

Figure 4 shows the osmolarity changes in interlobular space upon the addition of sucrose (300 mM) to the control perfusate. Fluid in the interlobular space was directly sampled by a pipet (10 μl). The osmolarity of the interlobular space was 301 mosM and upon the addition of sucrose it increased to 614 mosM within 4 min. The osmolarities measured show a good fit to an exponential curve, which was calculated by Eq. 2 (0.22 as α , difference of gland weight before and after blotting (0.71 g—interstitial fluid volume % of 71.3%) as V_{ECF} , and pump rate (2.4 ml/min) as F_v). The good fit of the theoretical curve indicates that this model describes adequately the changes in $[\text{OSM}]_{\text{ECF}}$ upon switching of the perfusate osmolarity to a new value. The good fit was also obtained in NaCl experiments (data not shown). This result shows that Eq. 2 is applicable to sucrose.

It is well-known that Cl^- is not confined only in interstitium. However, we used Cl^- as an indicator of interstitial osmolarity in this study. The reason we used

Fig. 4. Measured osmolarities of interstitial fluid and the theoretical curve derived using the model. The open circles represent measured osmolarities and the solid lines ($\tau=80.7$) are the theoretical curves calculated using Eq. 2. The perfusate osmolarity was changed from 301 mosM (solution E) to 615 mosM (solution F). Interstitial fluid of 10 μ l was sampled by a pipet and measured using an osmometer. The sampling intervals were 30–60 s. The pump rate was 2.4 ml/min. The value of V_{ECF} was 0.71 g. The value of α used was 0.22. The good fits of the theoretical curves indicate that the model describes adequately the changes in interstitial osmolarity upon switching the perfusate osmolarity to a new value

Cl^- is that Cl^- can be measured sensitively, continuously and easily by an Ag-AgCl electrode. It is well-known that the application of osmotic stress causes cell volumes to change [3, 10]. Submandibular acinar cells show a regulatory volume decrease (RVD) [11] and a regulatory volume increase (RVI) upon the application of osmotic stress. However, the RVD and RVI of the entire submandibular gland upon osmotic stress application are rather small compared with other types of cells (Nakahari, unpublished data [3, 10]). The ionic contents of the submandibular gland during hyperosmotic stress are reported to be almost unchanged as compared to those of a non-stressed submandibular gland [3]. This study indicates that the $[Cl^-]_{ECF}$, on the application of osmotic stress, was described by a simple model (Eq. 2) in which we assume that the net Cl^- flux from cells to interstitium is zero. These observations suggest that Cl^- may be a good indicator of the interstitial osmolarity in perfused submandibular glands.

Larcombe-McDouall *et al.* reported the interstitial fluid volume measured by 1H -NMR to be about 80% of a perfused submandibular gland (the perfusion rate was 2 ml/min); the interstitial fluid volume change upon osmotic stress (+60 mosM) application was negligibly small, but a change in the perfusion rate to 0.5 ml/min from 2 ml/min resulted in a change in the

interstitial fluid volume [10]. However, in our experiments, the interstitial volumes, which were calculated from weight difference of the gland before and after blotting, were approximately 60% of the entire gland weight at perfusion rates of 2.4 and 4.3 ml/min. At high perfusion rates (more than 2 ml/min), the changes in gland weight may be small as compared to those at a low perfusion rate (such as 0.5 ml/min). In our previous report, it was suggested that the submandibular gland still has about 15% of intralobular extracellular fluid remaining after blotting [6]. The total ECF volume of our experiments is calculated to be 75%, which is comparable to the interstitial fluid volume measured by 1H -NMR. This indicates that the weight difference of the gland before and after blotting is in proportion to the real interstitial fluid volume.

There are two possible explanations why value of α is not 1: the existence of a vascular shunt pathway and low permeability of the capillary walls. The values of α were independent of perfusion rates and gland weight and were almost constant. In the present experiments, we measured osmolarities of venous effluent. The changes in venous effluent after switching of the perfusate were 95% complete within 30 s, while those of interstitium required 180 s. As shown in electron micrographs, the capillaries were expanded in an artificially perfused submandibular gland [7]. This means that the perfusion pressure was high. A high perfusion pressure may cause opening of the shunt pathway. These observations suggest the existence of a shunt pathway. However, we do not know the exact answer to α at present. Furthermore, the effect of perfusate switching also affects the value of α , because the switching of perfusate osmolarity is not stepwise.

There have been some reports for determining the time constants of exchange between interstitium and capillaries in perfused organs [12] or whole body [13–15]. This study indicates that the value of α is constant under experimental conditions. This result may expand the applicability of Eq. 2. Thus, this method allows the determination of the time constant of interstitial fluid exchange from the interstitial fluid volume and perfusion rate, and allows the estimation of interstitial osmolarity following switching of the perfusate osmolarity in a perfused submandibular gland [5].

We thank Dr. Martin C. Steward (Manchester University) for useful suggestions and discussions. We also thank to Dr. M. Murakami (National Institute for Physiological Sciences) for his useful discussions and having the opportunity to perform some important experiments.

REFERENCES

1. Cook DI, van Lennep EW, Roberts ML, and Young JA: Secretion by the major salivary glands. *In: Physiology of the Gastrointestinal Tract*, 3rd ed., ed. Johnson LR, Raven Press, New York, pp 1061-1117, 1994
2. Case RM, Harper AA, and Scratcherd T: Water and electrolyte secretion by the perfused pancreas of the cat. *J Physiol (Lond)* 196: 133-149, 1968
3. Nakahari T, Yoshida H, Imai Y, Murakami M, and Seo Y: Osmotically inactive space during hyperosmotic stress in the perfused submandibular gland of rat. *Jpn J Physiol* 42: 977-985, 1992
4. Nakahari T, Nakamura A, and Steward MC: Paracellular fluid transport in salivary secretion? *In: Salivary Secretion: Control and Mechanisms*, International Workshop on Salivary Secretion, ed. Murakami M, Seo Y, and Ishikawa T, National Institute for Physiological Sciences, Okazaki, Japan, pp 81-84, 1992
5. Nakahari T, Steward MC, Yoshida H, and Imai Y: Osmotic flow transients during acetylcholine stimulation in the perfused rat submandibular gland. *Exp Physiol* 82: 55-70, 1997
6. Nakahari T, Yoshida H, Miyamoto M, and Imai Y: Measurement of extra-cellular fluid change in salivary gland using an impedance method. *Jpn J Physiol* 36: 565-583, 1986
7. Nakahari T, Nakamura A, and Imai Y: Two types of exocytosis evoked by ACh in salivary acinar cells: a morphological observation in perfused submandibular gland of rats. *Jpn J Physiol* 45: 23-36, 1995
8. Compton J, Martinez JR, Martinez AM, and Young JA: Fluid and electrolyte secretion from the isolated, perfused submandibular and sublingual glands of the rat. *Arch Oral Biol* 26: 555-561, 1981
9. Nakahari T, Murakami M, Yoshida H, Miyamoto M, Sohma Y, and Imai Y: Decrease in rat submandibular acinar cell volume during ACh stimulation. *Am J Physiol* 258: G878-G886, 1990
10. Larcombe-McDouall JB, Seo Y, and Steward MC: Continuous measurement of cell volume changes in perfused rat salivary glands by proton NMR. *Magnet Reson Med* 31: 131-138, 1994
11. Foskett JK, Wong MMM, Sue-A-Quan G, and Robertson MA: Isosmotic modulation of cell volume and intracellular ion activities during stimulation of single exocrine cells. *J Exp Zool* 268: 104-110, 1994
12. Murakami M, Seo Y, Matsumoto T, Ichikawa O, Ikeda A, and Watari H: Continuous measurements of Na, Li, and Cl in the perfused salivary gland by use of NMR. *Jpn J Physiol* 36: 1267-1274, 1986
13. Isogai Y, Nose H, Miki K, and Morimoto T: Dynamics of fluid movement between intravascular and interstitial spaces. *J Theor Biol* 100: 305-317, 1983
14. Ito T: Analysis of three compartments of extracellular fluid in dogs. *Jpn J Physiol* 34: 641-645, 1984
15. Nose H, Yamada S, and Morimoto T: Transvascular fluid shift and thoracic duct lymph: analysis of lymph formation in the rat. *Jpn J Physiol* 34: 713-729, 1984