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Role of the Main Mandibular Excretory Duct in Salivary Excretion of Urea in Dogs¹⁾

JUN WATANABE,* SATOSHI MIZUNO, KIKUO IWAMOTO
and SHOJI OZEKI

*Department of Biopharmaceutics, Faculty of Pharmaceutical Sciences,
Nagoya City University, 3-1, Tanabe-dori, Mizuho-ku,
Nagoya 467, Japan*

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The effect of passage through the main mandibular excretory duct on salivary excretion of urea was investigated using anesthetized dogs. Two kinds of mandibular saliva (pre-ductal saliva and post-ductal saliva) were collected from individual dogs. Pre-ductal and post-ductal mandibular saliva samples were collected individually *via* cannula tubing before and after flowing through the main mandibular excretory duct, respectively. Sublingual saliva was also collected to evaluate the effect of saliva passage through the non-striated duct. Salivation was evoked by electric nerve stimulation. The findings were as follows. (1) There was no significant difference between saliva/plasma concentration ratios (*S/P* ratios) of urea in the two mandibular salivas. (2) Salivary clearance of urea was not altered by the passage of saliva through the main mandibular excretory duct. (3) In this duct, sodium ion was slightly reabsorbed, but the *S/P* ratio of potassium ion was almost constant. Therefore, apparent water reabsorption may not take place in this duct. (4) From these results, it was concluded that the main mandibular excretory duct of the dog has no influence on salivary excretion of urea. (5) The mean *S/P* ratio of urea in sublingual saliva (1.25) was much higher than in mandibular saliva (0.330 to 0.345). Therefore, it is considered that urea might be either reabsorbed or diluted in the striated duct.

Keywords—urea; sodium; potassium; salivary excretion mechanism; mandibular saliva; main excretory duct; acinus; striated duct; salivary clearance; electric stimulation; sublingual saliva

Several review articles on salivary excretion of drugs or chemicals have appeared, mostly from a clinical point of view, during the last decade.²⁻⁵⁾ As regards the mechanism of the salivary transport of drugs or chemicals, it has been considered that they are excreted into saliva by a passive diffusion process obeying pH-partition theory (Matin's equation).⁶⁻⁸⁾ However, evidence that some drugs are actively secreted into the saliva cannot be explained by such a process.^{9,10)} Therefore, it was considered to be important to investigate the salivary excretion mechanism in detail to permit a rational assessment of the role of saliva in relation to pharmacokinetic studies and therapeutic drug monitoring.

In the preceding paper, we selected urea as a model compound for the above purpose and reported that salivary excretion of urea in dog parotid and mandibular-sublingual saliva could not be explained by Matin's equation. It was also found that at relatively low salivary flow rates, the saliva/plasma concentration ratios (*S/P* ratios) of urea increased for both salivas.¹¹⁾ In regard to these tendencies, other investigators proposed that urea might be concentrated due to water reabsorption driven by Na⁺ reabsorption in relatively low salivary flow rates in man, dog and rat parotid saliva.¹²⁻¹⁴⁾ However, there has been no experimental evidence to show whether salivary excretion of urea might be related to water or Na⁺ reabsorption, or whether the urea level in saliva might be modified during its flow from the acinus to the mouth.

The purpose of this work was to clarify whether urea and water transport occurs during

saliva flow through the main mandibular excretory duct in the dog. For comparison, sublingual saliva was also collected to evaluate the effect of saliva passage through the non-striated duct, since the sublingual gland of dog possesses no striated duct.¹⁵⁾

Experimental

Animals—Seven male mongrel dogs (No. 1, 7 kg; No. 2, 9 kg; No. 3, 13 kg; No. 4, 11 kg; No. 5, 8 kg; No. 6, 7 kg; No. 7, 15 kg) were used. Anesthesia was induced by intravenous administration of sodium pentobarbital (loading dose, 25 mg/kg; additional dose, 8 mg/kg) (Abbott Laboratories, Chicago, U.S.A.).

Operation and Cannulation—The main excretory ducts of the mandibular and sublingual gland, and the ramus communicans to mandibular ganglion were exposed on both sides of the dog. Two sites of cannulation were selected to collect two kinds of mandibular saliva (pre-ductal saliva, post-ductal saliva) in an individual dog, as illustrated in Fig. 1. The main excretory duct of the mandibular gland was cannulated at the glandular end (Fig. 1(A)) or oral end (Fig. 1(B)) with polyethylene tubing (PE-50 or PE-160, Natsume Seisakusho Co., Ltd. Tokyo, Japan) in five dogs (No. 1—5). In three dogs (No. 5—7), the main excretory duct of the sublingual gland was also cannulated at the glandular end. The ramus communicans to mandibular ganglion was ligated at the divarication from the lingual nerve to stimulate only the salivary gland. This nerve was kept moist with paraffin liquid.

Stimulation and Collection of Saliva and Plasma—The ramus communicans to mandibular ganglion was stimulated by using an electric nerve stimulator (DPS-05, Dia Medical System Co., Tokyo, Japan). Stimulus conditions were altered by varying the frequency from 5 to 100 Hz (5, 10, 20, 50, 100 Hz). The voltage (9 V) and pulse duration (3 ms) were kept constant. Under these stimulus conditions, an electric current of about 20 mA was passed to the nerve. A 10 min rest period was allowed between each stimulation. Because the first 10 drops of saliva was stagnant saliva present in the cannula during the resting period, this stagnant saliva was discarded. The pre-ductal or post-ductal saliva was then collected in four fractions (30 s each) in test tubes. However, the first 30-s saliva sample was not used for analysis, since it was considered to contain mostly stagnant saliva present in the gland before stimulation. Sublingual saliva was also collected in three dogs (No. 5—7) under fixed stimulus conditions (20 Hz, 9 V, 3 ms, about 20 mA). Salivation was stimulated intermittently for about 60 min to collect a sufficient volume of sublingual saliva. Blood samples were also collected from each dog, and plasma was obtained by centrifugation of the blood sample at 3000 rpm for 15 min.

Measurement of Salivary Flow Rate—The weight of each 30-s saliva sample instead of the volume was determined to estimate the salivary flow rate, assuming that the specific gravity of saliva is approximately 1.00.⁸⁾ Salivary flow rate was expressed as saliva volume per minute per body weight (ml/min/kg).

Determination of Urea—Urea concentrations in plasma and saliva were determined by the diacetyl monoxime method¹⁶⁾ using Urea N-Test Wako (Wako Pure Chemical Industries Ltd., Osaka, Japan).

Determination of Na⁺ and K⁺—Sodium and potassium ion concentrations in plasma and saliva were determined with a flame photometer (Shimadzu AA-630-12, Shimadzu Seisakusho Ltd., Kyoto, Japan).

Statistical Analysis—Inter-individual variations of salivary flow rates and *S/P* ratios were analyzed by means of analysis of variance, and were independent of the variation in these data. The significance of differences in the mean values of these data between pre- and post-ductal saliva was assessed by using Student's *t*-test.

Results and Discussion

S/P Ratios of Urea in Pre-ductal and Post-ductal Mandibular Saliva, and Salivary Flow Rates

In this study, mean values for *S/P* ratio of urea and salivary flow rate were obtained as summarized in Table I. There was no significant difference in *S/P* ratios of urea between pre-

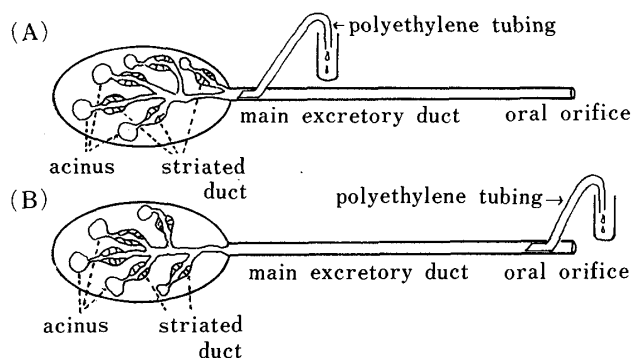


Fig. 1. Schematic Illustration of Sampling Sites of Pre-ductal and Post-ductal Mandibular Saliva and Structure of the Mandibular Gland

(A) Pre-ductal saliva: collected before flowing through the main mandibular excretory duct. (B) Post-ductal saliva: collected after flowing through the main mandibular excretory duct.

TABLE I. Saliva/Plasma Concentration Ratio of Urea and Salivary Flow Rate in Five Mongrel Dogs (No. 1—5)

	Pre-ductal saliva ^{a)} (<i>n</i> ^{c)} = 75)	Post-ductal saliva ^{b)} (<i>n</i> = 74)
S/P ratio ^{d)}	0.345 ± 0.105 ^{e)}	0.330 ± 0.0706
Salivary flow rate (ml/min/kg)	0.106 ± 0.0592	0.125 ± 0.0607

a) Collected before flowing through the main mandibular excretory duct. b) Collected after flowing through the main mandibular excretory duct. c) Number of data points. d) Saliva/plasma concentration ratio. e) S.D.

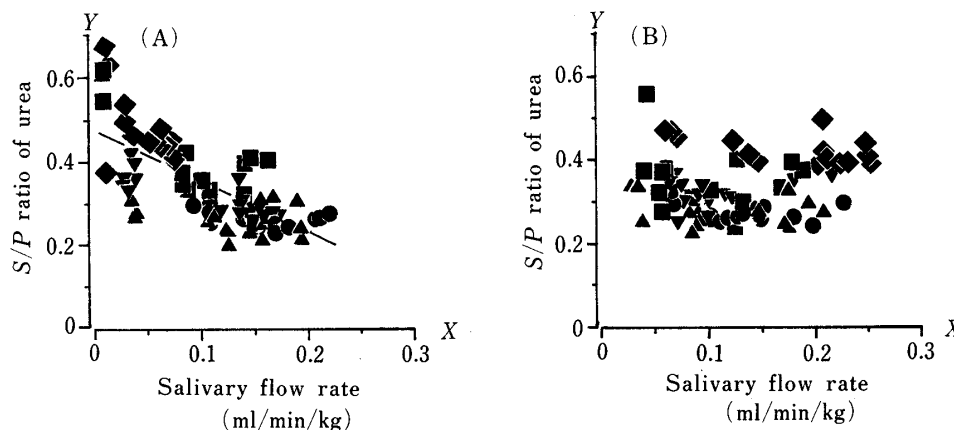


Fig. 2. Relationship between Saliva/Plasma Concentration Ratios of Urea and Salivary Flow Rates in Five Mongrel Dogs (No. 1—5)

Each shape of symbol represents an individual dog. (A) Pre-ductal saliva: see Fig. 1(A). The solid line shows the regression line (*n* = 75, *r* = -0.725, *Y* = -1.29*X* + 0.482, *p* < 0.01). (B) Post-ductal saliva: see Fig. 1(B).

TABLE II. Comparison of the Data for Saliva/Plasma Concentration Ratios of Urea in Different Ranges of Salivary Flow Rate in Five Mongrel Dogs (No. 1—5)

Range of salivary flow rate (ml/min/kg)	S/P ratios ^{a)}	
	Pre-ductal saliva ^{b)}	Post-ductal saliva ^{c)}
0—0.05	0.444 ± 0.131 ^{d)} (<i>n</i> ^{e)} = 18)	0.369 ± 0.114 (<i>n</i> = 5)
0.05—0.10	0.408 ± 0.0524 (<i>n</i> = 14)	0.323 ± 0.0648 (<i>n</i> = 27)
0.10—0.15	0.297 ± 0.0503 ^{f)} (<i>n</i> = 22)	0.308 ± 0.0616 (<i>n</i> = 19)
0.15—0.30	0.269 ± 0.0410 ^{f)} (<i>n</i> = 21)	0.348 ± 0.0703 (<i>n</i> = 23)
0—0.30	0.345 ± 0.105 ^{g)} (<i>n</i> = 75)	0.330 ± 0.0706 ^{g)} (<i>n</i> = 74)

a) Saliva/plasma concentration ratios. b) Collected before flowing through the main mandibular excretory duct. c) Collected after flowing through the main mandibular excretory duct. d) S.D. e) Number of data points. f) Significantly different from the data in the lowest flow rate range (0—0.05 ml/min/kg) at *p* < 0.001. g) The same value as listed in Table I.

ductal saliva and post-ductal saliva. Furthermore, it was considered that water transport in the main mandibular excretory duct did not occur during saliva flow through the duct, since there was no significant difference in salivary flow rate between the two salivas. The relationship between S/P ratios of urea and salivary flow rates was examined using individual data for pre-ductal saliva and post-ductal saliva and is illustrated in Fig. 2. As shown in Fig. 2(A), there was a significant negative correlation between S/P ratios of urea and salivary flow rates in pre-ductal saliva. This result for pre-ductal saliva (Fig. 2(A)) is similar to that for final mandibular-sublingual saliva in the preceding report.¹¹⁾ However, in post-ductal saliva (Fig. 2(B)), there was no significant correlation of S/P ratios of urea against salivary flow rates. This might be due to an insufficient number of data points in the low salivary flow rate range (0—0.05 ml/min/kg) under these experimental conditions. As it might be considered that the main mandibular excretory duct had some influence on S/P ratios of urea, the salivary flow rate was divided into four ranges for convenience. However, as shown in Table II, there was no significant difference between the mean values for the S/P ratios of urea in the two salivas. Therefore, this result also suggests that the S/P ratio of urea was scarcely altered during saliva flow through the main mandibular excretory duct at any salivary flow rate.

In the preceding paper, we introduced the concept of salivary clearance for stimulated salivary excretion of drugs or chemicals.¹¹⁾ In this work, we also considered the effect of saliva passage through the main mandibular excretory duct on the salivary clearance of urea.

Salivary Clearance of Urea and Salivary Flow Rate

In the preceding paper, we reported that salivary clearance of urea was highly dependent on salivary flow rate.¹¹⁾ As shown in Fig. 3(A) and 3(B), there was also a high correlation between salivary clearance of urea and salivary flow rate in both pre-ductal and post-ductal mandibular saliva samples. Since the slopes of these regression lines indicate that the S/P ratios of urea is constant, this result (Fig. 3(A)) was apparently inconsistent with the result in Fig. 2(A). However, in Fig. 2(A), the change of urea S/P ratio at relatively low flow rates was statistically significant but quite small. The above inconsistency is considered to arise because this change had almost no influence on the clearance of urea in relatively low flow rates and any effect was essentially cancelled by the variance of the data due to other factors.

As shown in Fig. 3, the slopes for pre-ductal saliva and post-ductal saliva were

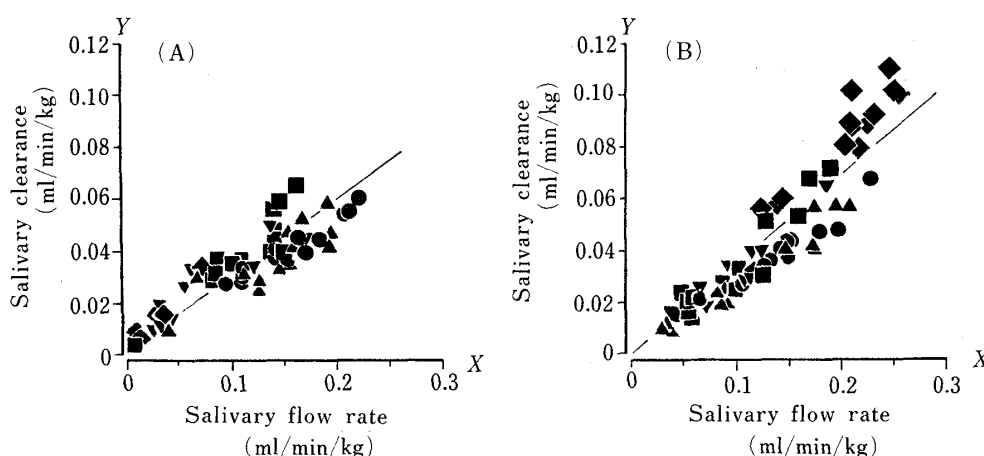


Fig. 3. Relationship between Salivary Clearances of Urea and Salivary Flow Rates in Five Mongrel Dogs (No. 1—5)

Each shape of symbol represents an individual dog. (A) Pre-ductal saliva: see Fig. 1(A). The solid line shows the regression line ($n=75$, $r=0.920$, $Y=0.289X$, $p<0.01$). (B) Post-ductal saliva: see Fig. 1(B). The solid line shows the regression line ($n=74$, $r=0.925$, $Y=0.342X$, $p<0.01$).

0.289 ± 0.00652 and 0.342 ± 0.00816, respectively. The difference in these slopes is not statistically significant, and thus the main mandibular excretory duct plays no major role in salivary excretion of urea, even if saliva flow or water transport in the duct is taken into account. In regard to water transport into the salivary gland, it is well known that water is driven into primary saliva by Na⁺ transport into the saliva.¹⁷⁻²⁰ Na⁺ and K⁺ transport was, therefore, examined in the main mandibular excretory duct from the point of view of water transport.

S/P Ratios of Na⁺ and K⁺ in Pre-ductal and Post-ductal Mandibular Saliva

It is known that in some experimental animals including the dog, Na⁺ is reabsorbed and K⁺ is secreted in the striated duct during saliva flow from the acinus to the mouth.¹⁷⁻²⁰ It has also been reported that Na⁺ is slightly reabsorbed, but water is not reabsorbed in perfused main parotid excretory duct of the rat.²¹ However, there is no report on ductal transport of Na⁺, K⁺ or water in dog main mandibular excretory duct. In this work, salivary flow rate was divided into four ranges (Table II). As shown in Table III, there was no significant difference between the mean values of *S/P* ratio of Na⁺ in the two kinds of saliva, in each salivary flow rate range. However, the two regression lines of *S/P* ratios of Na⁺ vs. salivary flow rates for the salivas intersected at 0.096 ml/min/kg, and at least at flow rates higher than 0.096 ml/min/kg, Na⁺ seemed to be slightly reabsorbed in the main mandibular excretory duct, since the slope of the regression line for post-ductal saliva (1.56) was significantly smaller than that for pre-ductal saliva (3.06). As shown in Table IV, however, the *S/P* ratio of K⁺ was nearly constant in every salivary flow rate range in both salivas. Further, in the same flow rate range there was also no significant difference between the mean *S/P* ratios of K⁺ in both salivas. Therefore, this result suggested that K⁺ was neither secreted nor concentrated (due to water reabsorption) in the main mandibular excretory duct.

As discussed above, it was concluded that the dog main mandibular excretory duct has no influence on the salivary excretion of urea. Therefore, it was considered that other parts of the

TABLE III. Comparison of the Data for Saliva/Plasma Concentration Ratios of Na⁺ in Different Ranges of Salivary Flow Rate in Five Mongrel Dogs (No. 1—5)

Range of salivary flow rate (ml/min/kg)	<i>S/P</i> ratios ^{a)}	
	Pre-ductal saliva ^{b)}	Post-ductal saliva ^{c)}
0—0.05	0.198 ± 0.143 ^{d)} (<i>n</i> ^{e)} = 18)	0.350 ± 0.254 (<i>n</i> = 5)
0.05—0.10	0.367 ± 0.224 ^{f)} (<i>n</i> = 14)	0.360 ± 0.186 (<i>n</i> = 27)
0.10—0.15	0.570 ± 0.124 ^{g)} (<i>n</i> = 22)	0.502 ± 0.177 ^{h)} (<i>n</i> = 19)
0.15—0.30	0.644 ± 0.0707 ^{g)} (<i>n</i> = 21)	0.599 ± 0.0901 ⁱ⁾ (<i>n</i> = 23)
0—0.30	0.463 ± 0.225 (<i>n</i> = 75)	0.478 ± 0.191 (<i>n</i> = 74)
Regression line ^{j)}	$Y = 3.06^k X + 0.138$	$Y = 1.56X + 0.282$

a) See Table II, footnote a). b) See Table II, footnote b). c) See Table II, footnote c). d) S.D. e) Number of data points. f) and g) Significantly different from the data in the lowest flow rate range (0—0.05 ml/min/kg) at *p* < 0.05 and at *p* < 0.001, respectively. h) and i) Significantly different from the data in the lower flow rate range (0.05—0.10 ml/min/kg) at *p* < 0.05 and *p* < 0.01, respectively. j) Regression line of *S/P* ratios (*Y*) vs. salivary flow rates (*X*). k) Significantly different from the data in the post-ductal saliva at *p* < 0.001.

TABLE IV. Comparison of the Data for Saliva/Plasma Concentration Ratios of K^+ in Different Ranges of Salivary Flow Rate in Five Mongrel Dogs (No. 1—5)

Range of salivary flow rate (ml/min/kg)	<i>S/P</i> ratios ^{a)}	
	Pre-ductal saliva ^{b)}	Post-ductal saliva ^{c)}
0—0.05	$2.09 \pm 0.302^d)$ (<i>n</i> ^{e)} = 18)	2.00 ± 0.103 (<i>n</i> = 5)
0.05—0.10	2.27 ± 0.357 (<i>n</i> = 14)	1.96 ± 0.483 (<i>n</i> = 27)
0.10—0.15	2.13 ± 0.521 (<i>n</i> = 22)	1.99 ± 0.473 (<i>n</i> = 19)
0.15—0.30	2.43 ± 0.492 (<i>n</i> = 21)	2.34 ± 0.391 (<i>n</i> = 23)
0—0.30	2.23 ± 0.453 (<i>n</i> = 75)	2.11 ± 0.429 (<i>n</i> = 74)

a) See Table II, footnote a). b) See Table II, footnote b). c) See Table II, footnote c). d) S.D. e) Number of data points.

TABLE V. Salivary Flow Rate, Saliva/Plasma Concentration Ratio of Urea, Na^+ and K^+ in Sublingual Saliva in Two (No. 5, 6) to Three (No. 5—7) Mongrel Dogs

Salivary flow rate (ml/min/kg)	<i>S/P</i> ratio ^{a)}		
	Urea	Na^+	K^+
$0.00031 \pm 0.00003^b)$ (<i>N</i> ^{d)} = 3)	1.25 ± 0.179 (<i>N</i> = 3)	$1.02 \pm 0.0771^c)$ (<i>N</i> = 2)	3.17 ± 0.276 (<i>N</i> = 2)

a) See Table II, footnote a). b) S.D. c) Range. d) Number of dogs.

mandibular gland such as the acinus and striated duct (see Fig. 1) might influence on the salivary excretion of urea. Thus, in this work sublingual saliva was collected for comparison, that is original saliva secreted in the acinus, to examine *S/P* ratios of urea in the acinus, since the sublingual gland had no striated duct and the electrolytes composition of the primary saliva is scarcely altered during saliva flow from the acinus to the mouth.¹⁵⁾ So far, there has been no report on *S/P* ratios of urea in dog sublingual saliva.

S/P Ratios of Urea in Sublingual Saliva

The *S/P* ratio of urea in sublingual saliva is listed together with the salivary flow rate and *S/P* ratios of Na^+ and K^+ in Table V. The mean value of *S/P* ratio of urea in sublingual saliva was about 1.25. The higher *S/P* ratio of urea as well as that of Na^+ in this saliva than in mandibular saliva might be related to the fact that the sublingual gland had no striated duct. Therefore, it is considered that relatively high *S/P* ratios of urea should exist in mandibular primary saliva and that the observed low *S/P* ratios of urea in mandibular saliva (0.330 or 0.345) probably result from reabsorption or dilution of urea in the striated duct.

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