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Difference in Saliva/Plasma Concentration Ratio of Endogenous Urea between Mandibular and Parotid Glands in Dogs[1)]

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The difference in saliva/plasma concentration ratio (S/P ratio) of urea between mandibular and parotid glands in dogs was investigated in detail by a technique of retrograde ductal injection of $HgCl_2$. In every dog, four kinds of saliva [untreated(control) mandibular and parotid saliva, $HgCl_2$-treated mandibular and parotid saliva] were collected under pilocarpine stimulation of salivation. The following results were obtained. (1) It was verified in this study that S/P ratios of urea and Na^+ were significantly increased in $HgCl_2$-treated mandibular saliva. (2) S/P ratios of urea and Na^+ in $HgCl_2$-treated parotid saliva were also significantly higher than those in untreated saliva. These results suggested that parotid salivary urea is also reabsorbed in the striated duct of dogs. (3) In untreated glands, the mean C_{Pr}/C_M ratio of urea (the ratio of urea concentration in parotid saliva to that in mandibular saliva) was about 1.65. This ratio tended to decrease toward unity in the $HgCl_2$-treated gland. Therefore, it was suggested that the striated duct might play an important role in the gland-specific difference of S/P ratios of urea between the salivary glands of dog.

Keywords——endogenous urea; salivary excretion mechanism; mandibular gland; parotid gland; striated duct; gland-specific difference; $HgCl_2$ retrograde injection; dog

Saliva levels of drugs or chemicals have been used for pharmacokinetic studies and in therapeutic drug monitoring.[3-6)] Regarding the salivary excretion mechanism of drugs or chemicals, it has been considered that most of them are excreted into saliva by a passive transport process obeying pH-partition theory (Matin's equation).[7,8)] In this laboratory, it has been reported that the observed values of saliva/plasma concentration ratios (S/P ratios) for indomethacin,[9)] phenobarbital,[10)] 5-fluorouracil[11)] and urea[12)] do not always agree with the estimated values according to Matin's equation, that a gland-specific difference in the S/P ratios is observed between parotid and mandibular–sublingual salivas of dogs, and that this difference can not be explained quantitatively by Matin's equation. 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.

With respect to salivary excretion of endogenous or exogenous urea, other investigators have studied only parotid saliva of man, dog and rat, and reported that the mean S/P ratio was about 0.7 but varied with the salivary flow rate.[13-15)] In our previous studies, salivary excretion of urea as a model compound has been studied using parotid,[12)] mandibular–sublingual,[12)] mandibular[16,17)] and sublingual[16)] saliva of dog. It was shown that: 1) S/P ratios of urea in parotid, mandibular–sublingual, mandibular and sublingual salivas were about 0.7, 0.45, 0.3 and 1.25, respectively, suggesting the existence of a gland-specific difference,[12,16)] 2) these results on S/P ratios of urea could not be explained by Matin's equation,[12)] 3) S/P ratios of urea in mandibular saliva are not altered during the salivary flow through the main mandibular excretory duct,[16)] 4) urea is reabsorbed in the striated duct of mandibu-

lar gland, and this reabsorption might be inhibited by the pre-treatment of the striated duct with $HgCl_2$.[17] These results suggested that the striated duct of mandibular gland might play a role in modifying the saliva concentration of urea and in the gland-specific difference of S/P ratios of urea.

In this work, therefore, the role of striated ducts of mandibular and parotid glands in the gland-specific difference of S/P ratios of endogenous urea was studied in detail by means of a retrograde ductal injection of $HgCl_2$ in dogs.[18,19]

Experimental

Animals——Four male mongrel dogs (No. 1, 6.0 kg; No. 2, 16.0 kg; No. 3, 10.0 kg; No. 4, 16.0 kg) were used. Anesthesia was induced by intravenous administration of sodium pentobarbital (Abbott Laboratories, Chicago, U.S.A.) at a loading dose of 25 mg/kg, and an additional dose of 8 mg/kg.

Operation and Cannulation——The main mandibular and parotid excretory ducts in both sides of the face were exposed and each duct was cannulated at the glandular end with polyethylene tubing (PE-50 or PE-160, Natsume Seisakusyo Co., Ltd., Tokyo, Japan). The experimental system is shown in Fig. 1.

Retrograde Injection of $HgCl_2$——In each dog, after saliva secretion *via* each cannula had been confirmed by pre-stimulation with pilocarpine, 0.05% $HgCl_2$ was injected retrogradely *via* the cannulae of the mandibular and parotid glands in one side of the face using a 1.0 ml syringe. The syringe was kept connected with the cannulae for 5 min ($HgCl_2$-treated glands). The other two glands were kept intact as the control glands (untreated glands) as shown in Fig. 1. The side for $HgCl_2$ treatment was randomized among four dogs. Ductal injection volume for the mandibular gland was 0.25—0.35 ml, since the approximate volume of the duct system, including striated duct, was considered to be in the range from 0.10 to 0.20 ml depending on the body weight of the dogs[18] and the dead space of the cannula was 0.15 ml in this experiment. In the parotid gland, ductal injection volume was 0.175—0.25 ml, since the approximate volume of the duct system, including striated duct, was similarly considered to be 0.075—0.15 ml[19] and the dead space of the cannula was 0.1 ml. Five min after the $HgCl_2$ injection, the injected solution was rapidly removed from the duct system of both salivary glands. Completeness of this removal was confirmed by the same method as reported by Henriques.[18]

Stimulation of Salivation and Collection of Biological Samples——Pilocarpine hydrochloride of JP grade (Hoei Yakko Co., Osaka, Japan) was used to induce salivation. Pre-stimulation was carried out to confirm saliva secretion *via* each cannula by i.v. bolus administration of the drug (0.015 mg/kg). About 15 min after the pre-stimulation, 0.15 mg/kg pilocarpine was injected intravenously to collect four kinds of saliva samples simultaneously (untreated mandibular and parotid salivas, $HgCl_2$-treated mandibular and parotid salivas). Saliva samples were collected in ten consecutive fractions for at 5-min intervals in test tubes. Blood samples were collected from the cephalic vein three times, before pre-stimulation, and at the midpoint and end point in each saliva sampling period. Plasma was obtained by centrifugation of the blood samples at 3000 rpm for 15 min.

Measurement of Salivary Flow Rate——The weight of the saliva sample, instead of the volume, was determined to estimate the salivary flow rate, assuming that the specific gravity of saliva was approximately 1.00.[10] The salivary flow rate was expressed as saliva volume per minute per body weight (ml/min/kg). Henriques[18] and Burgen[19] have reported that the retrogradely injected solution does not reach the acinus, and thus salivary flow rate is not altered by

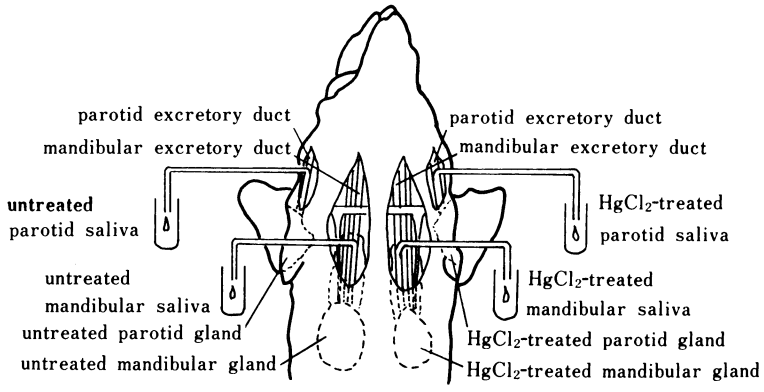

Fig. 1. Schematic Illustration of the Experimental System to Examine Salivary Excretion in Dog

TABLE I. Effect of Retrograde Injection of $HgCl_2$ on Salivary Flow Rate in Both Glands

Dog number	Salivary flow rate (ml/min/kg)			
	Mandibular saliva		Parotid saliva	
	Untreated saliva	$HgCl_2$-treated saliva	Untreated saliva	$HgCl_2$-treated saliva
No. 1 ($n^{a)}=10$)	$0.0477\pm0.0228^{b)}$	0.0417 ± 0.0291	0.0188 ± 0.00922	0.0216 ± 0.0130
No. 2 ($n=10$)	0.0444 ± 0.0498	0.0464 ± 0.0349	0.0184 ± 0.0225	0.0169 ± 0.0217
No. 3 ($n=10$)	0.122 ± 0.0595	0.123 ± 0.0765	0.0588 ± 0.0489	0.0583 ± 0.0531
No. 4 ($n=10$)	0.0680 ± 0.0626	0.0666 ± 0.0595	0.0242 ± 0.0300	0.0222 ± 0.0308
Total ($n=40$)	0.0704 ± 0.0538	0.0693 ± 0.0609	0.0300 ± 0.0344	0.0298 ± 0.0361

a) Number of data points. *b*) Mean ± S.D.

the injection. In this study, as shown in Table I, salivary flow rates were not altered by the treatment with $HgCl_2$ (assessed by the paired *t*-test).

Determination of Urea and Na^+——Endogenous urea is expressed simply as urea in this paper. Urea concentrations in saliva and plasma were determined by the diacetyl monoxime method[20] using Urea N-Test Wako (Wako Pure Chemical Industries Ltd., Osaka, Japan). Na^+ concentrations in the biological fluids were determined with an ion meter (IM 20E, Toa Electronic Ltd., Tokyo, Japan). During the experimental period, mean plasma concentration of urea and Na^+ in individual dogs ranged from about 0.200 to 0.365 mg/ml and from 120 to 165 μeq/ml, respectively, and the coefficients of variation % values of urea and Na^+ concentrations in individual dogs were about 3 to 5% and 8 to 11%, respectively. Thus, the mean plasma concentration of urea and Na^+ in each dog was used for the calculation of the S/P ratio in each dog.

Statistical Analysis——The significance of differences between C_{Pr}/C_M ratios of urea (the ratio of urea concentration in parotid saliva to that in mandibular saliva) in $HgCl_2$-treated and untreated saliva was assessed by Student's *t*-test, and for other data, the significance of differences was assessed by the paired *t*-test.

Results and Discussion

Effect of Retrograde Injection of $HgCl_2$ on S/P Ratios of Urea and Na^+ in Mandibular Saliva of Dog

It is well known that Na^+ is reabsorbed in the striated duct of salivary gland in dog, rabbit and rat,[18,19,21,22] and that $HgCl_2$ inhibits the Na^+ reabsorption.[18,19] In the preceding paper, we reported that urea was also reabsorbed in mandibular striated duct of dog, that the pre-treatment with $HgCl_2$ seemed to inhibit the reabsorption under electric nerve stimulation for salivation, and that the S/P ratio of urea in $HgCl_2$-treated mandibular saliva was significantly higher than that in untreated saliva.[17] In this work, as shown in Table II, S/P ratios of urea and Na^+ in $HgCl_2$-treated saliva were significantly higher than those in untreated mandibular saliva in every dog under pilocarpine stimulation of salivation. Therefore, it is suggested that the treatment with $HgCl_2$ markedly inhibits reabsorption of urea and Na^+ in the mandibular striated duct, under these two stimulus conditions for salivation.

It is well known that the parotid gland of dog also posesses the striated duct.[19,21,22] Thus, the effect of retrograde injection of $HgCl_2$ on the parotid gland was examined in the same dog.

Effect of Retrograde Injection of $HgCl_2$ on S/P Ratios of Urea and Na^+ in Parotid Saliva of Dog

There is no report demonstrating the effect of retrograde injection of $HgCl_2$ on parotid salivary excretion of urea. Regarding the effect of $HgCl_2$ treatment on parotid salivary excretion of Na^+, Burgen has reported that the treatment markedly inhibited reabsorption of Na^+ in parotid striated duct.[19] The results of our study on S/P ratios of urea and Na^+ in parotid saliva are listed in Table III. The S/P ratios of urea and Na^+ in $HgCl_2$-treated parotid

TABLE II. Effect of Retrograde Injection of $HgCl_2$ on S/P Ratio[a] of Urea and Na^+ in Mandibular Saliva

Dog number	S/P ratio			
	Urea		Na^+	
	Untreated saliva	$HgCl_2$-treated saliva	Untreated saliva	$HgCl_2$-treated saliva
No. 1 (n[c]=10)	0.485±0.103[d]	0.865[b]±0.172	0.0584±0.0372	0.787[b]±0.0769
No. 2 (n=10)	0.460±0.0498	0.922[b]±0.0795	0.474 ±0.0474	0.921[b]±0.0781
No. 3 (n=10)	0.385±0.0290	0.576[b]±0.0645	0.522 ±0.0132	0.674[b]±0.0864
No. 4 (n=10)	0.473±0.0712	0.593[e]±0.105	0.259 ±0.146	0.490[e]±0.124
Total (n=40)	0.449±0.0767	0.739[b]±0.191	0.328 ±0.212	0.718[b]±0.183

a) Saliva/plasma concentration ratio. *b*) Significantly different from the data for untreated saliva at $p<0.001$. *c*) Number of data points. *d*) Mean±S.D. *e*) Significantly different from the data for untreated saliva at $p<0.01$.

TABLE III. Effect of Retrograde Injection of $HgCl_2$ on S/P Ratio[a] of Urea and Na^+ in Parotid Saliva

Dog number	S/P ratio			
	Urea		Na^+	
	Untreated saliva	$HgCl_2$-treated saliva	Untreated saliva	$HgCl_2$-treated saliva
No. 1 (n[d]=10)	0.788±0.129[e]	0.942[b] ±0.0996	0.121±0.0532	0.524[c] ±0.0856
No. 2 (n=10)	0.780±0.172	0.856[f] ±0.112	0.663±0.197	0.950[c] ±0.0510
No. 3 (n=10)	0.653±0.0922	0.819[f] ±0.172	0.338±0.160	0.626[b] ±0.0531
No. 4 (n=10)	0.742±0.126	0.886[f] ±0.199	0.419±0.0986	0.982[c] ±0.0248
Total (n=40)	0.741±0.139	0.876[c] ±0.152	0.385±0.237	0.770[c] ±0.209

a) Saliva/plasma concentration ratio. *b*) Significantly different from the data for untreated saliva at $p<0.01$. *c*) Significantly different from the data for untreated saliva at $p<0.001$. *d*) Number of data points. *e*) Mean±S.D. *f*) Significantly different from the data for untreated saliva at $p<0.05$.

saliva were significantly higher than those in untreated saliva.

In regard to the parotid salivary excretion mechanism of urea, some investigators have proposed that urea is concentrated due to water reabsorption driven by Na^+ reabsorption in the striated duct of man, dog and rat parotid gland when the salivary flow rates are very low (in dogs, lower than approximately 0.05 ml/min/kg).[13-15] In this work, observed salivary flow rates were very low (approximately 0.02 to 0.06 ml/min/kg; Table I). It was expected that in $HgCl_2$-treated parotid saliva, urea would not be concentrated and the S/P ratios of urea should be lower than those in untreated saliva, since water reabsorption driven by reabsorption of Na^+ was expected to be substantially inhibited. However, the S/P ratios of urea in $HgCl_2$-treated saliva were slightly but significantly higher than those in untreated saliva (Table III). These results suggest that urea, like Na^+, is reabsorbed in the parotid striated duct of dog, and its reabsorption is inhibited by the treatment with $HgCl_2$. It was considered that the S/P ratio of urea in untreated parotid saliva of dog might be hardly influenced by the Na^+ reabsorption-driven water reabsoption (Table I), but the S/P ratio might be reduced in the striated duct due to the reabsorption of urea, as well as the S/P ratio of urea in untreated mandibular saliva of dog (Table II).

As discussed above, urea seemed to be reabsorbed in the striated duct of mandibular and parotid glands in dog. It was suggested that the striated duct of both salivary glands plays an important role in salivary excretion of urea. In this work, S/P ratios of urea in untreated

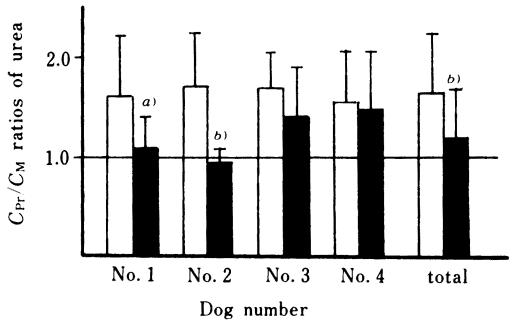

Fig. 2. Effect of Retrograde Injection of $HgCl_2$ on the C_{Pr}/C_M Ratio of Urea (the Ratio of Urea Concentration in Parotid Saliva to That in Mandibular Saliva)

The solid line shows the ratio of 1.0. ⊥, untreated saliva (mean ± S.D.); ▮, $HgCl_2$-treated saliva (mean ± S.D.). The number of data points in each dog is ten. *a*) and *b*) show significant differences from the data in untreated saliva at $p<0.05$ and $p<0.001$, respectively.

parotid saliva were higher than those in untreated mandibular saliva in each dog (Tables II, III). Thus, we next examined whether this gland-specific difference in S/P ratios of urea was related to the reabsorption of urea in the striated duct or not.

The Gland-Specific Difference in S/P Ratios of Urea between Mandibular and Parotid Glands of Dog

The C_{Pr}/C_M ratios of urea (the ratio of urea concentration in parotid saliva to that in mandibular saliva) in untreated and $HgCl_2$-treated salivas are shown in Fig. 2. C_{Pr}/C_M ratios of urea in untreated saliva in each dog ranged from 1.57 to 1.70 (total mean ratio; 1.65 ± 0.591, $n=40$). This ratio tended to decrease toward unity after the treatment with $HgCl_2$ in each dog (total mean ratio; 1.19 ± 0.512, $n=40$). These results suggest that the gland-specific difference of S/P ratios of urea between mandibular and parotid salivas was hardly observed before these salivas passed through the striated duct where the reabsorption of Na^+, water or urea itself would take place. It is, therefore, suggested that the striated duct of mandibular and parotid gland in dog may play an important role in the gland-specific difference of S/P ratio of urea between the salivary glands. Furthermore, it was considered that urea reabsorption in the striated duct of both salivary glands was a more important factor than water reabsorption driven by reabsorption of Na^+ for the gland-specific difference of S/P ratio of urea between mandibular and parotid glands of dog.

References and Notes

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