ROLE OF MANDIBULAR STRIATED DUCT IN SALIVARY EXCRETION OF UREA IN DOGS*

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(Received June 26, 1985)

The role of mandibular striated duct in salivary excretion of endogenous urea was studied by a technique of retrograde ductal injection of HgCl_2 and a stop flow analysis. (1) It was verified in this study that treatment with HgCl_2 markedly inhibited Na^+ reabsorption in the striated duct so that water reabsorption driven by the Na^+ reabsorption would be inhibited substantially. (2) The saliva/plasma concentration ratios (S/P ratios) of urea in HgCl_2 -treated saliva (about 0.71) were significantly higher than those in untreated saliva (about 0.37). (3) The excretion rate of urea in HgCl_2 -treated saliva (about 0.02 mg/min/kg) was also significantly higher than those in untreated saliva (about 0.01 mg/min/kg) despite the same flow rate for both saliva samples. Furthermore, (4) The S/P ratios of urea in the second (0.25 – 0.50 ml) poststop-flow saliva (about 0.65) were significantly higher than those in prestop-flow saliva (about 0.35). These results suggest a possibility that salivary urea is reabsorbed in the dog mandibular striated duct and that the enhancement of the S/P ratio as well as the salivary level of urea by the pre-treatment with HgCl_2 may be due to the inhibition of urea reabsorption.

Keywords — urea; salivary excretion mechanism; dog mandibular gland; mercuric chloride retrograde injection; stop flow analysis; mandibular striated duct

INTRODUCTION

Saliva levels of drugs or chemicals have been used for pharmacokinetic studies and in therapeutic drug monitoring.^{1–4)} Regarding the salivary excretion mechanism, it has been considered that most drugs and chemicals are excreted into saliva by a passive transport.⁵⁾ The theoretical equation (Matin's equation), which is based on pH-partition theory modified by drug protein binding in plasma and saliva, has been proposed to predict saliva/plasma concentration ratios (S/P ratios) of drugs or chemicals.⁶⁾ However, we have reported that the observed values of S/P ratios for indomethacin,⁷⁾ phenobarbital⁸⁾ and 5-fluorouracil⁹⁾ do not always agree with the values according to Matin's equation and that salivary excretion of these drugs is not explained quantitatively by the pH-partition theory.

With respect to salivary excretion of endogenous or exogenous urea, it has been reported that the mean S/P ratio in man, dog and rat parotid saliva were about 0.7, but varied with the salivary flow rate.^{10–12)} These results for S/P ratios of urea do not agree with the calculated value (almost 1.0) from Matin's equation.¹³⁾ It has

been suggested therefore, that for salivary excretion of drugs or chemicals, there may be some determining factors other than those defined in Matin's equation. We selected urea as one of the model compounds to clarify the major determinant in salivary excretion mechanism of drugs or chemicals in dogs and have reported as follows: 1) there was no significant difference between salivary excretion of endogenous and exogenous urea, 2) the gland specific difference between parotid and mandibular-sublingual saliva was found in the S/P ratios of urea, 3) the S/P ratio varied with the salivary flow rates,¹³⁾ and 4) the main mandibular excretory ducts played no significant role in the salivary excretion of urea. Thus, some possible roles of the mandibular striated duct was then proposed.¹⁴⁾ Although the striated duct has been regarded as a site influencing the salivary levels of some electrolytes such as Na^+ or K^+ ,^{15,16)} there are no reports demonstrating the role of mandibular striated duct in salivary excretion of urea.

In this paper, the role of the mandibular striated duct in salivary excretion of endogenous urea was studied in detail by means of a retrograde in-

* This paper constitutes Part VII of series entitled "Salivary excretion of drugs."

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traductal injection of HgCl_2 and a stop flow analysis in dogs.

MATERIALS AND METHODS

Animals — Nine male mongrel dogs (No. 1, 11.0 kg; No. 2, 13.5 kg; No. 3, 10.0 kg; No. 4, 10.0 kg; No. 5, 7.0 kg; No. 6, 7.0 kg; No. 7, 8.0 kg; No. 8, 8.5 kg; No. 9, 8.5 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 mandibular excretory ducts and ramus communicans to mandibular ganglion in both sides of the face were exposed and the ducts were cannulated at the glandular end with a polyethylene tubing (PE-50 or PE-160, Natsume Seisakusho Co., Ltd., Tokyo, Japan).

Retrograde Injection of HgCl_2 (Dog No. 1–5) — In each dog, 0.05% HgCl_2 solution in distilled water was injected retrogradely into one of the paired mandibular glands (HgCl_2 -treated gland) *via* the cannula, and the other mandibular gland was kept as the control gland (untreated gland). The side of HgCl_2 -treatment was randomized among five dogs. Ductal injection volume was fixed at 0.4 ml, since the approximate intraductal volume, except for the acinar space, in mandibular glands of mongrel dogs (about 12–15 kg) has been reported as 0.2 ml¹⁵⁾ and the dead space of the cannula was about 0.2 ml in this experiment. An injected solution was allowed to remain for 5 min, and the solution was rapidly removed from the duct system by salivation. Completeness of this removal was confirmed by the same method as reported previously.¹⁶⁾ HgCl_2 -treated saliva and untreated (control) saliva were collected from HgCl_2 -treated gland and non-treated gland, respectively. Both saliva samples were collected in three consecutive fractions (30 s each) in test tubes under electric nerve continuous stimulus conditions for 1.8 to 3.5 min, after the first 0.4 ml stagnant saliva was discarded. The ramus communicans to mandibular ganglion was directly stimulated for salivation by an electric nerve stimulator (S-7272B, Nihon Kodan, Tokyo, Japan). Stimulus conditions were adjusted by varying the frequency from 1 to 20 Hz (1, 2, 5, 10, 20 Hz). Electric current (20 mA) and pulse duration (3 ms) were kept constant. A 10-min rest period was allowed

between each stimulation. Blood samples were collected from the cephalic vein and plasma was obtained by centrifugation of the blood samples at 3000 rpm for 15 min.

Stop Flow Analysis (Dog No. 6–9) — Stop flow analysis was carried out as described below in untreated mandibular gland under the fixed stimulus condition (5 Hz, 3 ms, 20 mA) for 4.4 to 6.5 min. After collection of 0.4 ml saliva as the control (prestop-flow saliva sample) at a stabilized flow rate, the cannula was occluded for 2 min. The occlusion was then released and 4 or 6 consecutive fractions of saliva samples (poststop-flow saliva samples) were collected (about 0.25 ml each). The same process after a 10-min rest was repeated three times. Plasma was obtained in the same manner as described above.

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 1.00.⁸⁾ The salivary flow rate was expressed as saliva volume per min per body weight (ml/min/kg).

Determination of Urea and Na^+ — Endogenous urea is expressed simply as urea in this paper. Urea concentrations in plasma and saliva were determined by the diacetyl monoxime method¹⁷⁾ using Urea N-Test Wako (Wako Pure Chemical Industries Ltd., Osaka, Japan). Under this experimental condition, plasma concentration of endogenous urea in individual dog was found to be constant (Dog No. 1, 0.578 ± 0.0694 mg/ml; No. 2, 0.254 ± 0.00058 mg/ml; No. 3, 0.279 ± 0.0110 mg/ml; No. 4, 0.280 ± 0.0104 mg/ml; No. 5, 0.306 ± 0.00757 mg/ml; No. 6, 0.507 ± 0.0283 mg/ml; No. 7, 0.379 ± 0.00707 mg/ml; No. 8, 0.213 ± 0.0156 mg/ml; No. 9, 0.348 ± 0.0165 mg/ml). Na^+ concentrations were determined with a flame photometer (Shimadzu AA-630-12, Shimadzu Seisakusho Ltd. Kyoto, Japan) or ion meter (IM 20E, TOA Electronics Ltd., Tokyo, Japan).

Statistical Analysis — Significance of difference was assessed by the paired *t*-test.

RESULTS AND DISCUSSION

Effect of Retrograde Injection of HgCl_2 on Salivary Excretion of Urea

In regards to the salivary excretion mechanism of urea, some investigators have proposed that urea is concentrated due to water reabsorp-

tion driven by Na^+ reabsorption in the striated duct of man, dog and rat parotid glands.¹⁰⁻¹²⁾ The present experimental condition for treatment with HgCl_2 was considered to inhibit Na^+ reabsorption in the dog mandibular striated duct as reported previously.¹⁵⁾ As shown in Table I, the S/P ratio of Na^+ in HgCl_2 -treated saliva was significantly higher than that in untreated saliva in any dog. Therefore, it was expected that in HgCl_2 -treated saliva, urea would not be concentrated and the S/P ratios of urea might be lower than those in the untreated saliva, since water reabsorption driven by Na^+ reabsorption is supposed to be substantially inhibited under this experimental condition as indicated above (Table I). However, the mean S/P ratios of urea in HgCl_2 -treated saliva were significantly higher than those in untreated saliva (Table II). Fig. 1 shows the results of the S/P ratios of urea in untreated and HgCl_2 -treated saliva of dog No. 5. The S/P ratio of urea in HgCl_2 -treated saliva seemed to be always higher than that in the untreated saliva at all of the salivary flow rates measured. The same results were observed in the four other dogs. Furthermore, as shown in Table

III, the salivary excretion rates of urea in HgCl_2 -treated saliva were significantly higher than those in untreated saliva. In addition, there was no significant difference in the mean salivary flow rate between both salivas (untreated saliva; 0.118 ± 0.103 ml/min/kg, HgCl_2 -treated saliva; 0.0916 ± 0.0630 ml/min/kg), suggesting that the extent of Na^+ -mediated water reabsorption in the striated duct is small and that it has no apparent effects on the salivary flow rate. From these results, it was considered that Na^+ -mediated water reabsorption appeared to have no positive effects on salivary excretion of urea in dog mandibular glands. This consideration is not consistent with previous reports on the salivary excretion of urea in parotid glands.¹⁰⁻¹²⁾ This gland specific difference in the apparent role of water reabsorption in the striated duct would be considered to be responsible for the glandular difference in the S/P ratios of urea as was reported previously.¹³⁾

It was assumed that the significant increase in the S/P ratios of urea in HgCl_2 -treated saliva might be due to the enhanced urea secretion and/or the reduced urea reabsorption rather than

TABLE I. Effect of Retrograde Injection of HgCl_2 on S/P Ratios ^{a)} of Na^+

	S/P ratio of Na^+					
	No. 1 ^{b)}	No. 2	No. 3	No. 4	No. 5	Total
Untreated saliva	0.236 $\pm 0.179^c)$ ($n=15$) ^{d)}	0.372 ± 0.338 ($n=15$)	0.287 ± 0.216 ($n=15$)	0.402 ± 0.361 ($n=15$)	0.271 ± 0.299 ($n=15$)	0.312 ± 0.287 ($n=75$)
HgCl_2 -treated saliva	0.497 ^{e)} ± 0.0808 ($n=15$)	0.733 ^{e)} ± 0.0724 ($n=15$)	0.908 ^{e)} ± 0.0511 ($n=15$)	0.828 ^{e)} ± 0.0800 ($n=15$)	0.844 ^{e)} ± 0.0462 ($n=15$)	0.761 ^{e)} ± 0.159 ($n=75$)

^{a)} Saliva/plasma concentration ratios. ^{b)} Dog No. ^{c)} Mean $\pm$ S.D. ^{d)} Number of data points. ^{e)} Significantly different from the data for untreated saliva at $p < 0.001$.

TABLE II. Effect of Retrograde Injection of HgCl_2 on S/P Ratios ^{a)} of Urea

	S/P ratio of urea					
	No. 1 ^{b)}	No. 2	No. 3	No. 4	No. 5	Total
Untreated saliva	0.324 $\pm 0.0506^c)$ ($n=15$) ^{d)}	0.478 ± 0.0703 ($n=15$)	0.419 ± 0.0685 ($n=15$)	0.233 ± 0.0652 ($n=15$)	0.401 ± 0.189 ($n=15$)	0.371 ± 0.131 ($n=75$)
HgCl_2 -treated saliva	0.704 ^{e)} ± 0.134 ($n=15$)	0.764 ^{e)} ± 0.0440 ($n=15$)	0.773 ^{e)} ± 0.0683 ($n=15$)	0.489 ^{e)} ± 0.0325 ($n=15$)	0.805 ^{e)} ± 0.0986 ($n=15$)	0.707 ^{e)} ± 0.141 ($n=75$)

See Table I, footnotes a—e).

to any inhibitory effect of Na^+ -mediated water reabsorption in the striated duct by this treatment. Therefore, even in the control mandibular gland (untreated gland), the salivary urea levels could be expected to be altered by its secretion or reabsorption in the striated duct. Stop flow analysis was then carried out in the untreated gland to determine whether urea was secreted or reabsorbed in the striated duct.

Effect of Stop Flow on Salivary Excretion of Urea

The stop flow analysis technique in dog mandibular gland has been reported previously by Henriques and has been summarized as follows¹⁶⁾: 1) the first poststop-flow saliva sample comes from most of the ducts and a few acini, whereas the second sample comes from proximal segments of the ducts and acini, and 2) the highest Na^+ concentration is observed in the second sample, which is interpreted as acinar secretion, since during the occlusion period, the striated duct possesses minimal Na^+ reabsorption. There-

fore, if urea as well as Na^+ is reabsorbed in the striated duct, the highest concentration should be observed in the poststop-flow saliva sample in which the highest Na^+ concentration is observed.

The results of the experiment on the effect of stop flow on S/P ratios of Na^+ and urea presented in Fig. 2 are from dog No. 7. Similar results were also observed in three other dogs. As shown in Fig. 2, the highest S/P ratio of Na^+ was observed in the second poststop-flow saliva sample (0.25–0.50 ml). The results indicate that the striated duct has a relatively small Na^+ reabsorption effect in this sample, as reported previously.¹⁶⁾ The highest S/P ratio of urea was also observed in this saliva sample. These values for the S/P ratios of urea and Na^+ were significantly higher than those in the prestop-flow saliva. These results suggested that there was some reabsorption process for urea as well as Na^+ in the dog mandibular striated duct. However, the urea reabsorption mechanism in the striated duct appeared to be different from the active Na^+ reabsorption mechanism, since the S/P ratio of Na^+ with the highest value in the second cumulative poststop-flow sample returned readily to the prestop-flow ratio in succeeding samples as compared with the relatively slow return of the urea S/P ratio to the prestop-flow ratio (Fig. 2). In addition, it is possible that the retrograde HgCl_2 -treatment may have some inhibitory effects on the urea reabsorption mechanism in the striated duct, which would be different from the inhibitory effects on the Na^+ reabsorption mechanism.

In regards to the transport pathway for urea, water and Na^+ in biological membranes, it has been reported that the transport pathway in red

FIG. 1. *S/P Ratios of Urea in Untreated and HgCl_2 -Treated Saliva (Dog No. 5)*

Open symbols represent the S/P ratios in untreated saliva. Solid symbols represent the S/P ratios in HgCl_2 -treated saliva.

TABLE III. *Effect of Retrograde Injection of HgCl_2 on Salivary Excretion Rate of Urea*

	Salivary excretion rate of urea (mg/min/kg)					
	No. 1 ^{a)}	No. 2	No. 3	No. 4	No. 5	Total
Untreated saliva	0.0125 ± 0.00813 ^{b)} (<i>n</i> = 15) ^{c)}	0.0124 ± 0.00956 (<i>n</i> = 15)	0.00819 ± 0.00402 (<i>n</i> = 15)	0.00980 ± 0.00713 (<i>n</i> = 15)	0.0152 ± 0.00713 (<i>n</i> = 15)	0.0116 ± 0.00845 (<i>n</i> = 75)
HgCl_2 -treated saliva	0.0224 ^{d)} ± 0.0132 (<i>n</i> = 15)	0.0187 ^{d)} ± 0.0118 (<i>n</i> = 15)	0.0191 ^{e)} ± 0.00839 (<i>n</i> = 15)	0.0129 ^{d)} ± 0.00781 (<i>n</i> = 15)	0.0260 ^{d)} ± 0.0201 (<i>n</i> = 15)	0.0198 ^{e)} ± 0.0134 (<i>n</i> = 75)

a) Dog No. b) Mean $\pm$ S.D. c) Number of data points. d) Significantly different from the data for untreated saliva at $p < 0.01$. e) Significantly different from the data for untreated saliva at $p < 0.001$.

FIG. 2. Typical Comparison of S/P Ratios of Urea and Na⁺ in Poststop-Flow Saliva with Those in Prestop-Flow Saliva (Dog No. 7)

Solid columns represent mean values of S/P ratios for urea. Open columns represent mean values of S/P ratios for Na⁺. Vertical bars show the standard deviation ($n = 3 - 6$). a) Significantly different from the data for prestop-flow saliva at $p < 0.01$. b) Significantly different from the data for prestop-flow saliva at $p < 0.05$.

blood cells and toad bladders are inhibited by the treatment of Hg²⁺ or amiloride: but these pathways are different from each other.^{18,19)} These reports appear to support our consideration as discussed above, despite the different types of biological membranes. In this work, reabsorption mechanism of urea in the striated duct has not been investigated in detail. Therefore, further work to elucidate the urea reabsorption mechanism in the striated duct and urea transport in the acinus are being conducted in this laboratory.

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