Taste of a Pill

ORGANIC CATION TRANSPORTER-3 (OCT3) MEDIATES METFORMIN ACCUMULATION AND SECRETION IN SALIVARY GLANDS*

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Background: Drug excretion into saliva has important clinical implications, but the mechanisms underlying salivary gland drug transport remain unclear.
Results: OCT3 is highly expressed in secretory epithelial cells and mediates active metformin transport in salivary glands.
Conclusion: OCT3 provides a unique pathway for metformin secretion and accumulation in salivary glands.
Significance: Carrier-mediated salivary secretion can provoke drug-induced taste disturbance.

Drug-induced taste disturbance is a common adverse drug reaction often triggered by drug secretion into saliva. Very little is known regarding the molecular mechanisms underlying salivary gland transport of xenobiotics, and most drugs are assumed to enter saliva by passive diffusion. In this study, we demonstrate that salivary glands selectively and highly express OCT3 (organic cation transporter-3), a polyspecific drug transporter in the solute carrier 22 family. OCT3 protein is localized at both basolateral (blood-facing) and apical (saliva-facing) membranes of salivary gland acinar cells, suggesting a dual role of this transporter in mediating both epithelial uptake and efflux of organic cations in the secretory cells of salivary glands. Metformin, a widely used anti-diabetic drug known to induce taste disturbance, is transported by OCT3/Oct3 *in vitro*. *In vivo*, metformin was actively transported with a high level of accumulation in the salivary glands of wild-type mice. In contrast, active uptake and accumulation of metformin in salivary glands were abolished in $Oct3^{-/-}$ mice. $Oct3^{-/-}$ mice also showed altered metformin pharmacokinetics and reduced drug exposure in the heart. These results demonstrate that OCT3 is responsible for metformin accumulation and secretion in salivary glands. Our study uncovered a novel carrier-mediated pathway for drug entry into saliva and sheds new light on the molecular mechanisms underlying drug-induced taste disorders.

Many medications, including antihypertensive, anti-diabetic, antimicrobial, and antidepressant drugs, have the potential to adversely influence a patient's sense of taste and smell (1, 2). In some cases, medications can produce prolonged taste disturbance that cannot be quickly reversed by drug cessation (1). Taste-related adverse effects can significantly impact a patient's quality of life, dietary choices, emotional state, and compliance with medication regimens, especially in pediatric and geriatric populations (1–3). As taste receptors are located

in taste buds on the tongue, the physical presence of a drug substance in saliva is a prerequisite for taste disturbance caused by many medications. Drugs are known to be excreted into saliva; however, other than passive diffusion, the molecular mechanisms by which salivary gland epithelial cells secret xenobiotics are virtually unknown (4, 5).

One drug known to be excreted into saliva and to cause taste disturbance is metformin, a first-line oral hypoglycemic agent used in the treatment of type 2 diabetes. Patients on metformin therapy frequently experience a lingering metallic taste in the mouth evidently due to a persistent presence of metformin in the saliva (6, 7). In humans after either oral or intravenous dosing, metformin is readily detectable in the saliva (8). The concentration of metformin in saliva is lower than in blood, but declines much more slowly (8). When administrated to mice, metformin was reported to accumulate in several tissues and organs, including salivary glands (9). Metformin is a hydrophilic and positively charged molecule that relies on transporters to move across cell membranes (10, 11). Metformin is a substrate of multiple polyspecific organic cation transporters, including OCT1–3 (organic cation transporter-1–3), MATE1 and MATE2-K (multidrug and toxin extrusion protein 1 and 2-K), and PMAT (plasma membrane monoamine transporter) (10–12). In humans, the liver-specific OCT1 mediates metformin uptake into the liver, which is a primary site of metformin pharmacological action (10, 11). Recent studies have shown that OCT1 is important for metformin therapeutic response and that genetic polymorphisms in *OCT1* may contribute to variation in clinical response to the drug (13). Metformin undergoes negligible metabolism *in vivo* and is eliminated predominantly by the kidney (11). The kidney-specific OCT2 works in concert with MATE1 to mediate renal secretion of metformin (10). OCT3 and PMAT, two newer polyspecific organic cation transporters with a broad tissue distribution, also transport metformin *in vitro* (12, 14). However, their roles in metformin disposition and action *in vivo* are less clear.

In this study, we determined the expression and cellular localization of OCT3/Oct3 in the salivary glands of humans and mice. The *in vivo* significance of Oct3 in salivary gland drug

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transport was evaluated in mice with targeted deletion of the *Oct3/Slc22a3* gene.

EXPERIMENTAL PROCEDURES

Animals—The *Oct3* (*Slc22a3*) null mice of the FVB inbred strain were originally developed by Dr. Denise Barlow (15) and maintained by Dr. Alfred Schinkel (Netherlands Cancer Institute). After re-derivation at Charles River Laboratories (16), breeding pairs of *Oct3$^{+/+}$* and *Oct3$^{-/-}$* mice were kindly provided to us by Dr. John Markowitz (University of Florida) with approval from Dr. Schinkel. These mice were housed in the specific pathogen-free facility at the University of Washington. All animal studies were approved by the Institutional Animal Care and Use Committee of the University of Washington.

Metformin Transport Assay—The full-length mouse Oct3 (mOct3)[2] cDNA was amplified from mouse brain cDNA and confirmed to fully align with the reference sequence NM_011395.2. Flp-In HEK293 cells stably expressing mOct3 was generated following a similar procedure as described previously for human OCT3 (17). Metformin uptake was determined by radiotracer uptake assay using [^{14}C]metformin as described previously (12, 17). Cells stably transfected with pcDNA5/FRT vector were used as a control, and transporter-specific uptake was calculated by subtracting uptake in control cells from uptake in transporter-expressing cells. Metformin transport kinetic studies were carried out at varying substrate concentrations, and kinetic parameters were determined in the initial uptake phase as described previously (17).

mRNA and Protein Analysis in Mouse and Human Tissues—For mRNA determination in various mouse tissues, 10–12-week-old *Oct3$^{+/+}$* and *Oct3$^{-/-}$* mice were used ($n = 4$). Mouse liver, kidney, small intestine, heart, salivary gland, and skeletal muscle tissues were collected, flash-frozen in liquid N_2, and stored at $-80\,°C$ until analysis. For mRNA analysis in human salivary glands, total RNA (Clontech) pooled from 24 male and female Caucasians (16–60 years old) was used. For regional OCT3 mRNA and protein analysis in human parotid, submandibular, and sublingual glands, post-mortem tissues from a single female donor (30 years old) with no disease history were provided by the National Disease Resource Interchange. Total RNA was extracted, and TaqMan real-time PCR was performed on an Applied Biosystems 7900HT fast real-time PCR system as described previously (18). To determine OCT3 membrane protein levels, total membrane proteins were isolated from the human parotid, submandibular, and sublingual glands using the ProteoExtract native membrane protein extraction kit (Calbiochem). The membrane fraction was digested by trypsin, and quantification of OCT3 protein was carried out using a recently developed quantitative LC-MS/MS method on an Agilent 6460A triple-quadrupole mass spectrometer coupled to the Agilent 1290 Infinity LC system (18).

Immunofluorescence Labeling of OCT3/Oct3 in Human and Mouse Salivary Glands—Snap-frozen human submandibular gland was processed to frozen sections and fixed in ice-cold acetone. The sections were blocked in goat serum in PBS, incu-

bated overnight at $4\,°C$ with rabbit anti-OCT3 polyclonal antibody (1:125 dilution; Genway), and co-labeled with anti-human Na$^+$/K$^+$-ATPase α-subunit monoclonal antibody (1:500 dilution; Sigma). The sections were then washed with PBS, and fluorescent tag-labeled secondary antibodies (1:500 dilution; Alexa 488 for OCT3 and Alexa 568 for Na$^+$/K$^+$-ATPase) were applied for 1 h at room temperature. For a nonspecific control, tissue sections of human submandibular gland were incubated with blank serum, followed by secondary antibody incubation under the same conditions. For Oct3 immunolocalization in mouse salivary glands, frozen tissue sections were prepared from *Oct3$^{+/+}$* and *Oct3$^{-/-}$* mice and immunostained with rabbit anti-Oct3 polyclonal antibody (1:125 dilution), followed by incubation with Alexa 488-tagged secondary antibody (1:500 dilution). After washing, ProLong Gold antifade medium with DAPI was mounted on the slides and cover-slipped. Fluorescent images were obtained with a Zeiss Axiovert 200 fluorescence microscope (19).

In Vivo Study in Mice—Because rodent salivary glands exhibit significant sexual dimorphism (20), *in vivo* studies were performed in two separate cohorts of male and female mice. Male and female mice (10–12 weeks old) were fasted for ~10 h. Age- and gender-matched *Oct3$^{+/+}$* and *Oct3$^{-/-}$* mice were administered 15 mg/kg metformin containing 0.2 mCi/kg [^{14}C]metformin by oral gavage. At various time points (0–480 min), mice (n = three to six mice at each time point) were killed by CO_2 overdose, followed by cardiac puncture. Blood was collected using a heparin-coated syringe, and plasma was separated by centrifugation at $5000 \times g$. Tissues, including liver, kidney, skeletal muscle, heart, salivary gland, and intestinal segments, were collected and processed at each time point using the method described by Wilcock and Bailey (9). Plasma and tissue metformin concentrations were determined by liquid scintillation counting. Metformin concentration was expressed as nanograms/g for tissues and nanograms/ml for plasma.

Pharmacokinetic Data Analysis—Metformin plasma concentration-time curves were plotted and analyzed. Because metformin concentrations in plasma and tissue were sampled in different animals at each time point (one-point sampling), a population-based bootstrap method (21, 22) was used to calculate the mean and confidence intervals of the plasma and tissue area under the concentration-time curves (AUCs) and other pharmacokinetic parameters, including oral clearance (CL/F), terminal half-life ($t_{1/2,\beta}$), and volume of distribution at terminal phase (V/F), using Equations 1–4.

$$\text{AUC}_{0-t} = \int_0^t C(t)dt \qquad \text{(Eq. 1)}$$

$$\frac{\text{CL}}{F} = \frac{\text{dose}}{\text{AUC}_{0-\infty}} \qquad \text{(Eq. 2)}$$

$$t_{1/2,\beta} = \frac{\ln 2}{\beta} \qquad \text{(Eq. 3)}$$

$$\frac{V}{F} = \frac{\text{CL}}{F \cdot \beta} \qquad \text{(Eq. 4)}$$

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[2] The abbreviations used are: mOct3, mouse Oct3; AUC, area under the curve; C_{max}, peak plasma concentration.
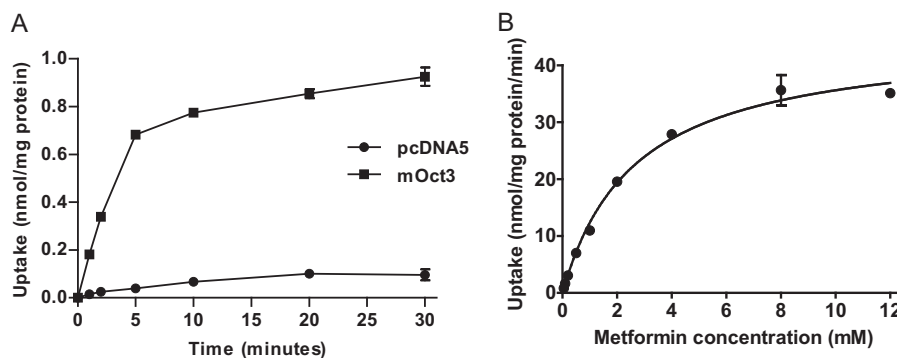
FIGURE 1. **Mouse Oct3 transports metformin.** *A*, time-dependent uptake of metformin (10 μM) in mOct3-expressing HEK293 cells and pcDNA5-transfected (control) cells. *B*, metformin transport kinetics by mOct3. Uptake was performed with varying metformin concentrations for 5 min in HEK293 cells stably transfected with mOct3. Transporter-specific uptake was calculated by subtracting the transport activity in control cells.

The terminal slope (β) was calculated by performing a linear regression of concentrations at the last three to five time points. The 95% confidence intervals for pharmacokinetic parameters were generated using the bootstrap method with modification (21, 22). Briefly, plasma or tissue concentrations were resampled with random replacement of the total number of mice using the R program. The resampling was repeated 10,000 times to create 10,000 pseudo concentration-time profiles. For each profile, the concentrations at each nominal time point were averaged, and the pharmacokinetic parameters were calculated using Equations 1–4. The 95% confidence intervals for each parameter were calculated by taking the 2.5 and 97.5% quartiles (*i.e.* the 251th and 97,501th values of 10,000 bootstrap values). Peak plasma concentration (C_{max}) was estimated by visual inspection of the plasma concentration-time curve.

Statistical Analysis—Quantitative assays were performed in duplicate or triplicate and repeated at least two times. Data are presented as mean ± S.D., unless specified otherwise. For pharmacokinetic data (except C_{max}), 95% confidence intervals around the estimate for each pharmacokinetic parameter were determined using the non-parametric bootstrap method (21). Two-sided p values were calculated using permutation tests with 10,000 replications (23). For comparison of C_{max} and non-pharmacokinetic parameters, statistical significance was determined by unpaired Student's t test. A p value <0.05 was considered statistically significant.

RESULTS

mOct3 Efficiently Transports Metformin—Metformin is known to be an excellent substrate of human OCT3 (14, 24). To confirm that mOct3 also transports metformin, we examined metformin uptake by mOct3 using Flp-In HEK293 cells stably expressing mOct3. Metformin uptake was much higher in mOct3-expressing cells, and mOct3-mediated metformin uptake exhibited typical saturable kinetics (Fig. 1, *A* and *B*). The apparent affinity (K_m) was 2.65 ± 0.23 mM, and the maximal transport rate (V_{max}) was 45.1 ± 1.4 nmol/mg of protein/min. These data suggest that similar to human OCT3, mOct3 also efficiently transports metformin.

Salivary Glands Predominantly Express OCT3—Previous gene profiling studies reported a wide distribution of OCT3 mRNA in various human tissues, with major expression in skeletal muscle, cardiac myocytes, placenta, liver, adrenal gland,

prostate, and salivary glands (14, 25). As Oct3 expression was less studied in mice, we first quantified mRNA expression of Oct3 and other known metformin transporters, including Oct1, Oct2, Mate1, and Pmat, in various mouse tissues relevant to metformin disposition and response (Fig. 2). As expected, mouse liver predominantly expressed Oct1 and Mate1, and mouse kidney predominantly expressed Oct1, Oct2, and Mate1. In the small intestine, Oct1 and Mate1 prevailed in all segments, although Oct3 and Pmat were also significantly expressed in the ileum. Mouse heart and skeletal muscle had a very high expression of Mate1 together with a significant expression of Oct3. Among the mouse tissues analyzed, the highest expression of Oct3 mRNA was observed in the salivary glands (Fig. 3A), whereas the mRNA of other organic cation or biogenic amine transporters (Sert (serotonin transporter), Net (norepinephrine transporter), and Dat (dopamine transporter)) was minimally expressed (Fig. 3B). A similar predominant expression of OCT3 mRNA was observed in human salivary glands (Fig. 3C). Further analysis revealed that OCT3 mRNA was expressed in all three major types of human salivary glands, with the highest expression found in submandibular glands (Fig. 3D). Using a targeted quantitative proteomics method (18), OCT3 protein in human parotid, submandibular, and sublingual glands was quantified to be 0.42 ± 0.11, 1.53 ± 0.015, and 0.43 ± 0.29 fmol/μg of total membrane protein, respectively.

OCT3/Oct3 Protein Is Localized to Both Basolateral and Apical Membranes of Salivary Gland Epithelial Cells—To examine the membrane localization and cell type-specific expression of OCT3/Oct3 in human and mouse salivary glands, immunohistochemistry was carried out with cryostat tissue sections using a previously validated polyclonal antibody against OCT3 (14). In human submandibular glands, robust immunoreactivity for OCT3 was observed on acini and associated ducts (Fig. 4A). There was essentially no staining when the primary anti-OCT3 antibody was substituted with blank serum (data not shown). In acinar cells (the secretory epithelial cells responsible for producing saliva), OCT3 immunostaining not only superimposed with the basolateral membrane marker Na^+/K^+-ATPase but also extended to the apical membranes lining the canaliculi (Fig. 4A). A similar pattern of both basolateral and apical immunostaining was observed in the salivary glands of $Oct3^{+/+}$ mice
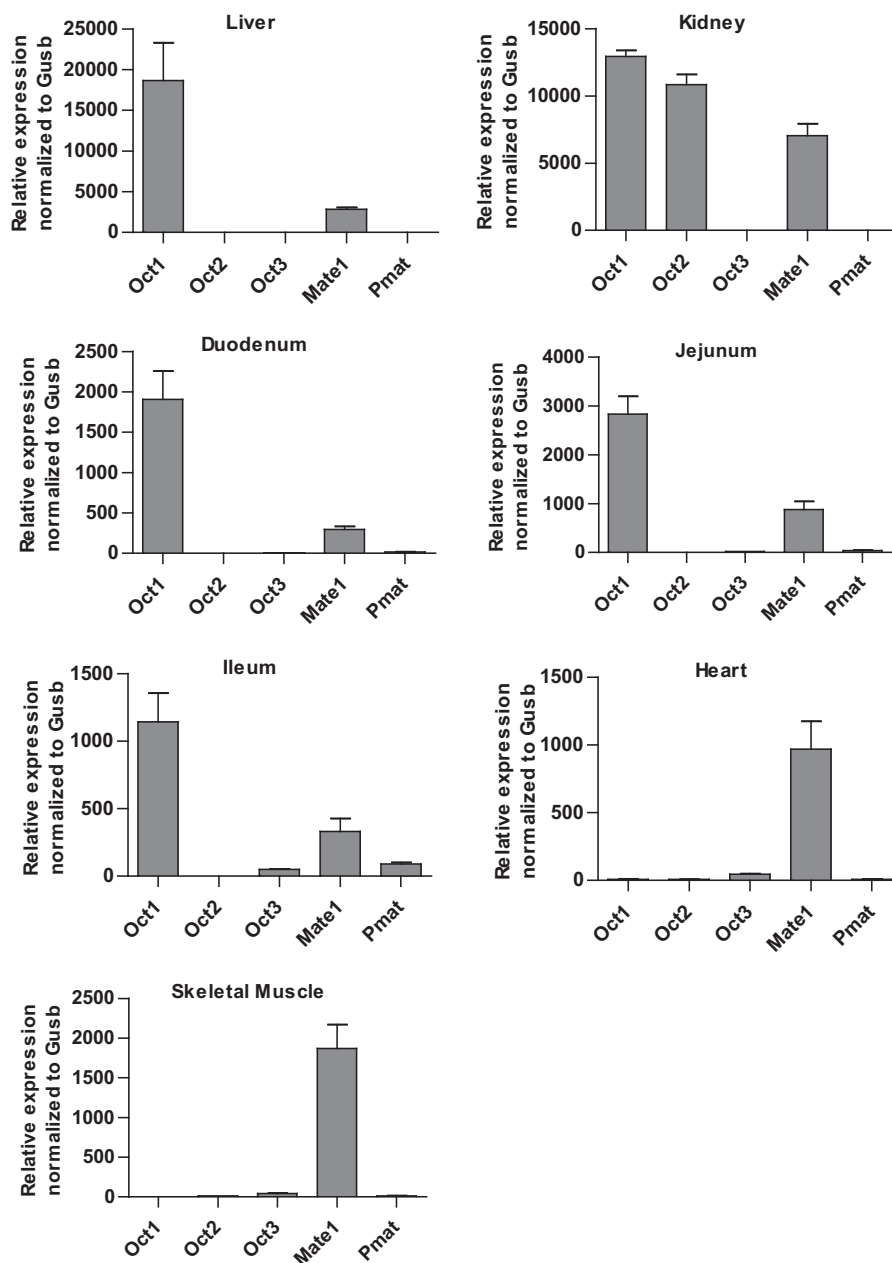
FIGURE 2. **Expression of Oct1–3, Mate1, and Pmat mRNAs in various mouse tissues determined by quantitative RT-PCR.** Transporter expression was normalized to the expression of the mouse β-glucuronidase (Gusb) gene.

(Fig. 4*B*). In contrast, no specific basolateral or apical staining was observed in the salivary glands of $Oct3^{-/-}$ mice (Fig. 4*B*). Together, these data revealed that the OCT3/Oct3 protein is present on both basolateral (blood-facing) and apical (saliva-facing) membranes of the secretory epithelial cells in human and mouse salivary glands, suggesting a multifaceted role of this transporter in mediating solute transport in salivary gland epithelial cells.

$Oct3^{-/-}$ Mice Exhibit Altered Plasma Pharmacokinetics of Metformin—Prior to *in vivo* studies, we confirmed by quantitative RT-PCR that there were no significant compensatory changes in the expression of other metformin transporters (Oct1, Oct2, Mate1, Pmat) in the tissues of interest in $Oct3^{-/-}$ mice (data not shown). The plasma concentration-time profiles and pharmacokinetic parameters of metformin in female and male

$Oct3^{+/+}$ and $Oct3^{-/-}$ mice are shown in Fig. 5 and Tables 1 and 2. Although the overall systemic exposure ($AUC_{0-480\,min}$) was comparable between $Oct3^{+/+}$ and $Oct3^{-/-}$ mice (Tables 1 and 2), a clear difference was observed in metformin plasma concentrations in the first 60 min between the two genotypes (Fig. 5). In female $Oct3^{-/-}$ mice, the C_{max} of metformin increased by 67%, and $AUC_{0-60\,min}$ increased by 41% (Table 1). A similar change in the metformin pharmacokinetic profile was observed in male $Oct3^{-/-}$ mice (Table 2). These data suggest that Oct3 influences metformin pharmacokinetics and has a significant impact on metformin plasma levels in the early phase following drug administration.

Metformin Accumulation Is Greatly Reduced in the Salivary Glands of $Oct3^{-/-}$ Mice—In $Oct3^{+/+}$ mice, the highest metformin tissue exposure (defined by $AUC_{0-480\,min}$) was observed
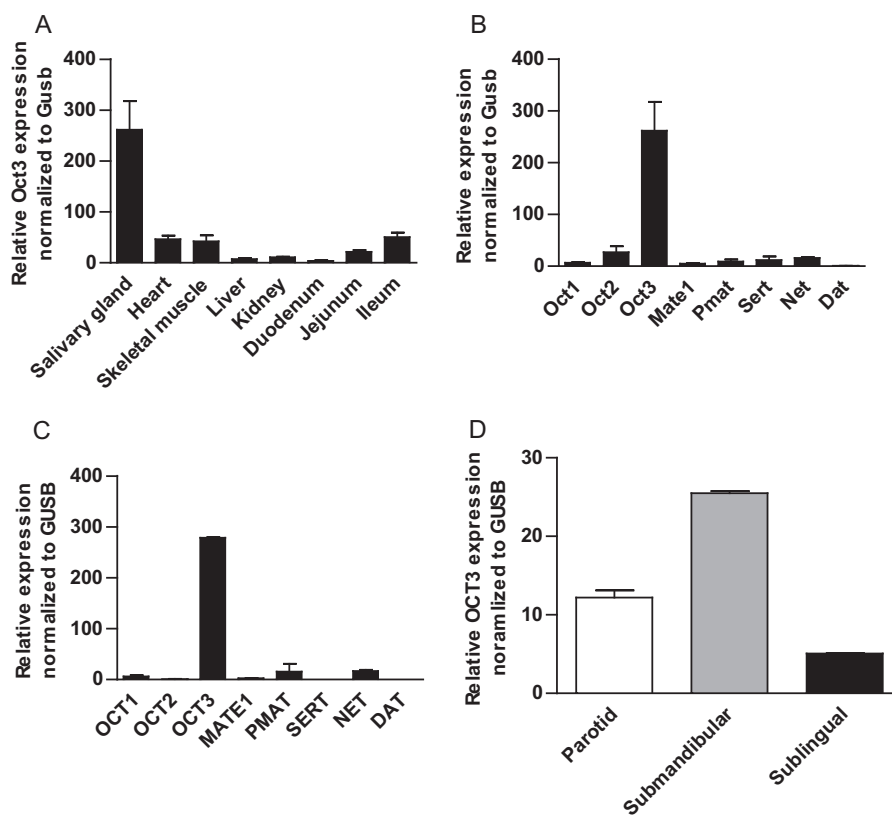
FIGURE 3. **Salivary glands predominantly express OCT3/Oct3.** *A*, relative expression of mOct3 mRNA in various mouse tissues determined by quantitative RT-PCR. *B* and *C*, gene profiling of functionally related transporters in mouse (*B*) and human (*C*) salivary glands by quantitative RT-PCR. *D*, OCT3 mRNA expression in human parotid, submandibular, and sublingual glands. The results were normalized to the expression of the human or mouse β-glucuronidase (GUSB/Gusb) gene.

in the kidney and salivary glands, followed by the liver (Table 3). Metformin concentrations were much lower in the salivary glands of $Oct3^{-/-}$ mice at all time points after 10 min (Fig. 6*A*). At 60 min, the average metformin concentration in the salivary glands of $Oct3^{-/-}$ mice was only one-sixth of that in wild-type mice. The overall metformin exposure ($AUC_{0-480\,min}$) in salivary glands was reduced by 64 and 54% in female and male $Oct3^{-/-}$ mice, respectively (Table 3). These data strongly suggest that Oct3 actively accumulates metformin from the blood in the salivary glands. The concentrative nature of this process was evident when metformin concentration-time profiles were compared between salivary glands and plasma. In wild-type mice, metformin salivary gland tissue concentrations were 2–4-fold higher than plasma concentrations at most time points (Fig. 6*B*). The intracellular metformin concentrations in salivary gland epithelial cells are expected to be even greater if adjusted to intracellular water volume. In contrast, this concentrative feature was absent in $Oct3^{-/-}$ mice, as metformin salivary gland concentrations rarely surpassed plasma concentrations in these animals (Fig. 6*C*).

Consistent with significant expression of Oct3 in the heart (Fig. 3*A*), metformin $AUC_{0-480\,min}$ in this tissue was significantly reduced by 33 and 34% in female and male $Oct3^{-/-}$ mice, respectively (Table 3). In the skeletal muscle of $Oct3^{-/-}$ mice, metformin exposure appeared to be reduced in both genders, and the reduction was statistically significant in male mice. Liver metformin exposure appeared to be increased in $Oct3^{-/-}$

mice, and the increase in male mice was statistically significant. The reason for this observation is unclear but could be due to an increased early plasma drug exposure, which may allow more Oct1-mediated metformin uptake into the liver. No change was observed in the kidney. Tissue exposure in the gastrointestinal track could not be accurately measured due to a significant contamination of unabsorbed metformin remaining in the intestinal lumen.

DISCUSSION

Drug-induced taste disorders are common adverse effects that provoke medication compliance issues especially in children and elderly patients. Drugs are known to be excreted into saliva, which represents the first step for taste disturbance induced by many medications. Sampling drug concentrations in saliva is clinically used in the assessment of therapeutic levels of drugs and the monitoring of illicit drug use (4, 5). Nevertheless, very little is presently known regarding the molecular mechanisms underlying salivary secretion of drugs and other foreign chemicals. Most drugs have been assumed to enter saliva by passive diffusion, a process characterized by downhill, non-mediated diffusion of drug molecules across the membranes of the secretory epithelial cells in salivary glands (4). However, passive diffusion can neither explain salivary secretion of hydrophilic drugs (*e.g.* metformin) nor account for high drug accumulation in salivary glands. Our study revealed a critical role of OCT3/Oct3 in metformin transport in salivary
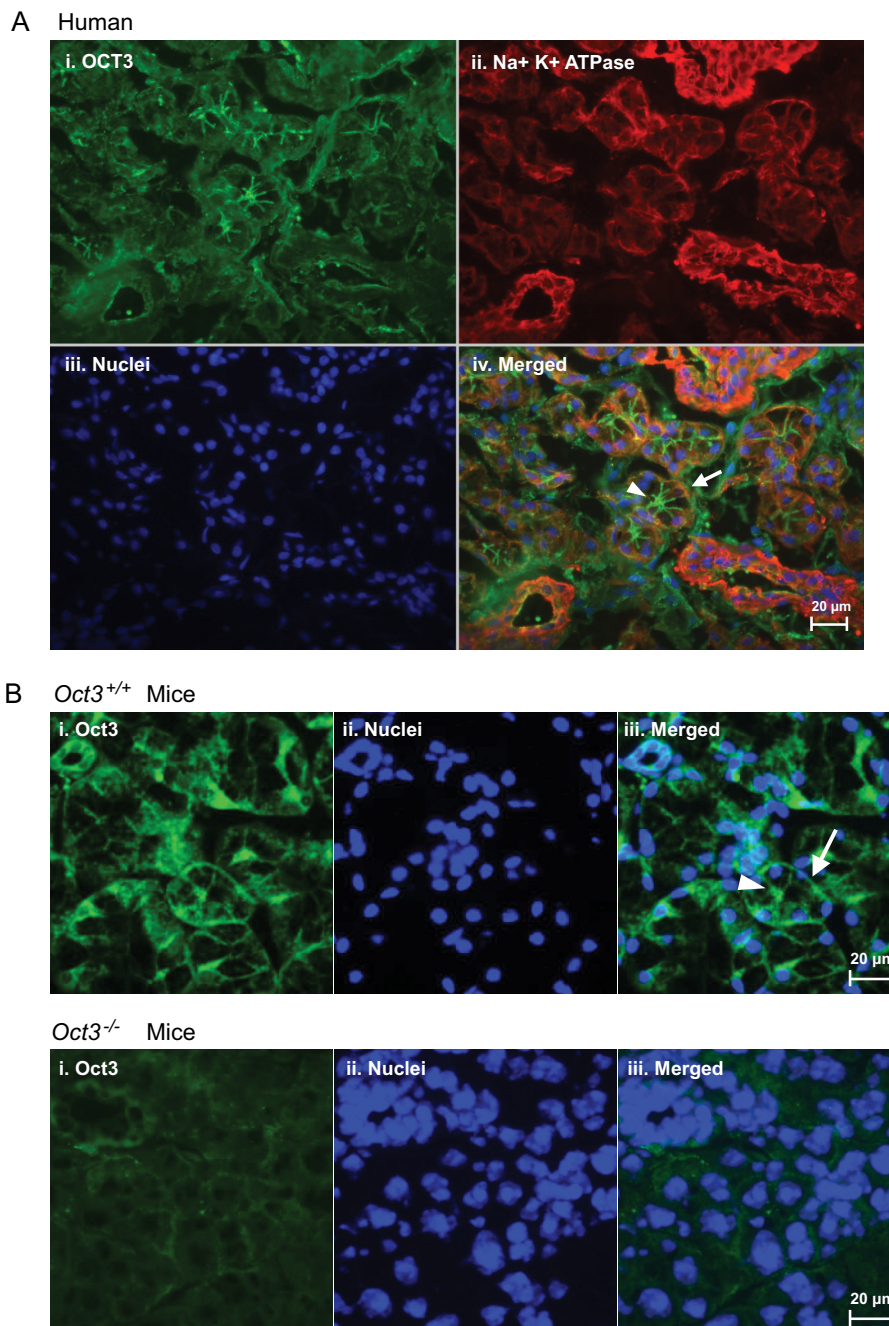
⨑ASBMB

FIGURE 4. **Localization of OCT3/Oct3 in salivary gland epithelial cells.** *A*, detection of OCT3 (*panel i*, *green*) in human submandibular glands. Staining of Na$^+$/K$^+$-ATPase (*panel ii*), a basolateral marker, and nuclei (*panel iii*) is shown in *red* and *blue*, respectively. *B*, detection of Oct3 (*panel i*, *green*) and nuclei (*panel ii*, *blue*) in salivary gland sections from *Oct3*$^{+/+}$ (*upper panels*) and *Oct3*$^{-/-}$ (*lower panels*) mice. In overlays (*A*, *panel iv*, and *B*, *panel iii*), the *arrow* and *arrowhead* indicate basolateral and apical membranes of salivary gland epithelial cells, respectively.

glands and provided a molecular mechanism for metformin secretion into saliva and the associated long-lasting taste disturbance effect. To our knowledge, this is the first time that a carrier-mediated mechanism has been demonstrated for drug accumulation and secretion in salivary glands.

OCT proteins are electrogenic bidirectional transporters that translocate their substrates down the electrochemical gradient (26, 27). Although OCT1 and OCT2 are expressed mainly in the liver and kidney, OCT3 is known to have a broader tissue distribution. Our data clearly show that OCT3/Oct3 is highly expressed in salivary glands, and the submandibular gland,

which is responsible for generating 70% of all saliva, has the highest OCT3 expression (Fig. 3). OCT3/Oct3 protein is localized to both basolateral (blood-facing) and apical (saliva-facing) membranes of salivary gland acinar cells (Fig. 4), suggesting a dual role of this transporter in metformin transport in these secretory epithelial cells. On the basis of these data, we propose a cellular model for OCT3 expression and transport function in salivary gland acinar cells (Fig. 7). The physiologic inside-negative electrical membrane potential difference (approximately −70 mV) in acinar cells provides a driving force for basolateral OCT3 to concentrate metformin from blood into acinar cells.
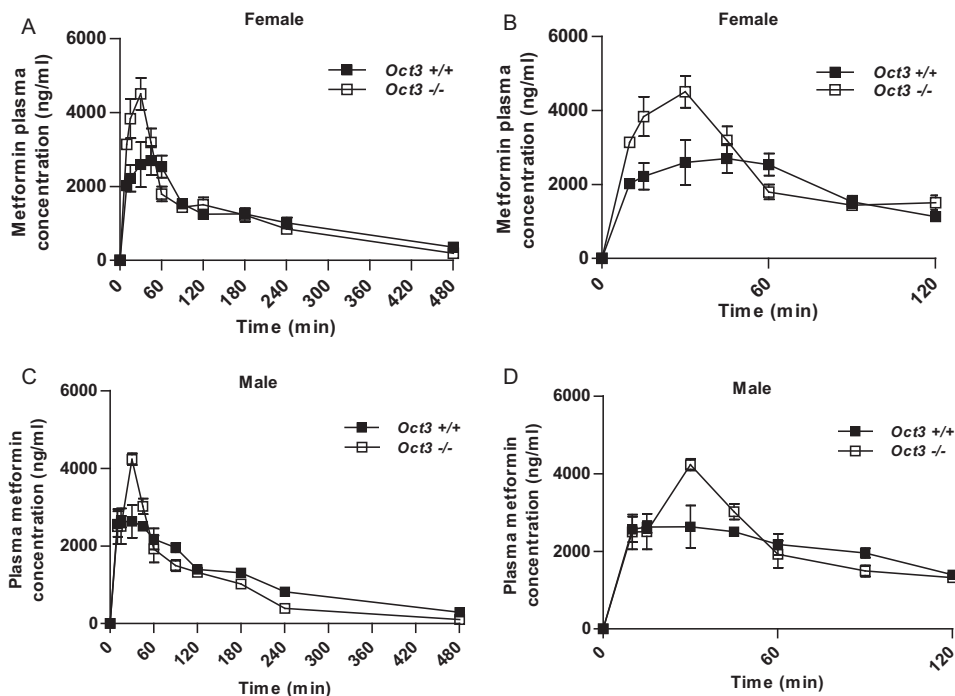
FIGURE 5. **Oct3 impacts metformin plasma kinetics.** *A* and *B*, plasma metformin concentration-time profiles from 0 to 480 min (*A*) and from 0 to 120 min (*B*) in female $Oct3^{+/+}$ and $Oct3^{-/-}$ mice. *C* and *D*, plasma metformin concentration-time profiles from 0 to 480 min (*C*) and from 0 to 120 min (*D*) in male $Oct3^{+/+}$ and $Oct3^{-/-}$ mice. Mice were given by oral gavage a dose of 15 mg/kg metformin containing 0.2 mCi/kg [^{14}C]metformin. Animals were killed at each time point, and the plasma and tissue concentrations of metformin were measured by liquid scintillation counting. Data represent mean $\pm$ S.E. (n = three to six mice at each time point). A total of 60 $Oct3^{+/+}$ and 53 $Oct3^{-/-}$ female mice and a total of 48 $Oct3^{+/+}$ and 40 $Oct3^{-/-}$ male mice were used for the pharmacokinetic studies.

TABLE 1

Metformin pharmacokinetic parameters in female $Oct3^{+/+}$ and $Oct3^{-/-}$ mice

Data are presented as mean $\pm$ S.D. (C_{max}) or with 95% confidence interval in parentheses.

Parameter	$Oct3^{+/+}$	$Oct3^{-/-}$	Change	p value
			%	
C_{max} (μg/ml)	2.70 $\pm$ 0.79	4.51 $\pm$ 0.86	↑ 67	0.037
$AUC_{0-60\ min}$ (μg*min/ml)	136 (119–155)	191 (170–211)	↑ 41	0.002
$AUC_{0-480\ min}$ (μg*min/ml)	546 (475–599)	553 (494–602)	↑ 1	0.862
CL/F (ml/min/kg)[a]	27.5 (25.0–31.6)	27.1 (24.9–30.4)	↓ 1	0.863
Terminal $t_{1/2}$ (min)	164 (126–284)	113 (89.4–142)	↓ 31	0.237
V/F (liters/kg)	6.51 (4.83–11.04)	4.42 (3.45–5.94)	↓ 32	0.230

[a] CL/F values were calculated using dose/$AUC_{0-480\ min}$.

TABLE 2

Metformin pharmacokinetic parameters in male $Oct3^{+/+}$ and $Oct3^{-/-}$ mice

Data are presented as mean $\pm$ S.D. (C_{max}) or with 95% confidence interval parentheses.

Parameter	$Oct3^{+/+}$	$Oct3^{-/-}$	Change	p value
			%	
C_{max} (μg/ml)	2.63 $\pm$ 0.32	4.23 $\pm$ 0.26	↑ 61	<0.001
$AUC_{0-60\ min}$ (μg*min/ml)	139 (124–154)	167 (145–181)	↑ 20	0.063
$AUC_{0-480\ min}$ (μg*min/ml)	529 (384–569)	433 (373–495)	↓ 18	0.052
CL/F (ml/min/kg)[a]	28.3 (26.4–39.1)	34.7 (30.3–47.4)	↑ 22	0.069
Terminal $t_{1/2}$ (min)	143 (114–182)	98.1 (71.2–124)	↓ 31	0.308
V/F (liters/kg)	5.85 (4.68–8.06)	4.91 (3.71–6.19)	↓ 16	0.619

[a] CL/F value was calculated using Dose/$AUC_{0-480min}$.

Initial metformin concentrations in saliva are very low, however, producing an outwardly directed electrochemical gradient of metformin at the apical membrane. This would drive apical OCT3 to efflux a small amount of metformin into the saliva, leading to its low but continuous presence in the saliva. Our hypothesis is supported by the *in vivo* data in mice showing Oct3-mediated uptake and high accumulation of metformin in salivary glands (Fig. 6). It would be interesting to compare metformin concentrations in the salivary fluids of $Oct3^{+/+}$ and $Oct3^{-/-}$ mice. However, we were not able to perform these experiments due to technical difficulties in collecting saliva samples in mice. Regardless, our cellular model, which indicates a low but continuous metformin secretion by apical Oct3, is in line with the clinical observation of the low but persistent presence of metformin in human saliva after drug administration (8). Although it is often anticipated that pairs of different, asymmetrically localized transporters are needed to mediate transepithelial solute transport, there are well established

TABLE 3

Tissue $AUC_{0-480\ min}$ from female and male $Oct3^{+/+}$ and $Oct3^{-/-}$ mice

Data are presented as the estimate, with 95% confidence interval in parentheses.

| Tissue	$Oct3^{+/+}$	$Oct3^{-/-}$	Change	*p* value
	µg*min/g	*µg*min/g*	%	
Salivary gland				
Female	1881 (1672–2087)	677 (593–757)	↓ 64	<0.001
Male	1375 (921–1509)	630 (422–772)	↓ 54	<0.001
Heart				
Female	389 (351–426)	262 (229–291)	↓ 33	<0.001
Male	317 (227–340)	208 (171–260)	↓ 34	<0.001
Skeletal muscle				
Female	318 (269–365)	286 (235–326)	↓ 10	0.344
Male	284 (146–318)	188 (122–223)	↓ 34	0.035
Liver				
Female	995 (849–1123)	1111 (983–1242)	↑ 12	0.263
Male	912 (730–1029)	1242 (1045–1412)	↑ 36	0.004
Kidney				
Female	1938 (1725–2143)	1761 (1486–2076)	↓ 9	0.328
Male	1770 (1215–1994)	1723 (1417–2015)	↓ 3	0.849
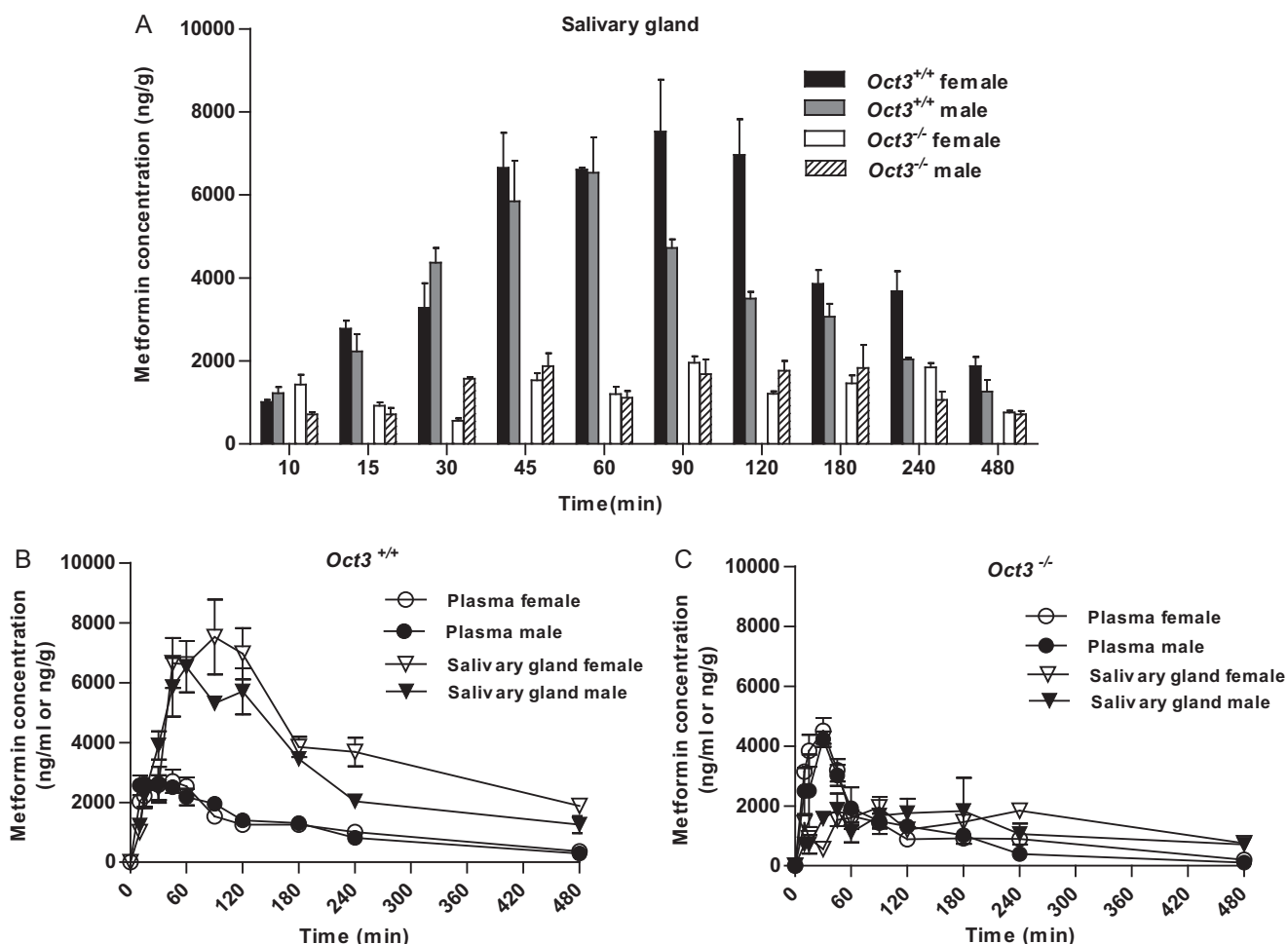
FIGURE 6. **Oct3 mediates metformin accumulation in salivary glands.** *A*, metformin concentrations in salivary glands at various time points (0–480 min) in female and male $Oct3^{+/+}$ and $Oct3^{-/-}$ mice. *B* and *C*, comparisons of plasma *versus* salivary gland metformin concentrations at various time points in $Oct3^{+/+}$ (*B*) and $Oct3^{-/-}$ (*C*) mice.

examples showing that a single transporter can be expressed at both membrane domains to facilitate transepithelial flux of a solute. For example, transport of glucose across the placental barrier is predominantly accomplished by a single bidirectional glucose transporter, GLUT1, which is expressed on both apical and basal membranes of placental epithelial cells to mediate maternal-to-fetal transport of glucose (28, 29). However,

despite the critical role of OCT3/Oct3 in salivary gland metformin transport as revealed by our *in vitro* and *in vivo* data, low but significant expression of other metformin transporters (*e.g.* Oct1, Oct2, and PMAT) was observed in mouse and/or human salivary glands (Fig. 3). These transporters may also influence metformin transport in salivary glands. Further studies are needed to determine the membrane localization of these trans-
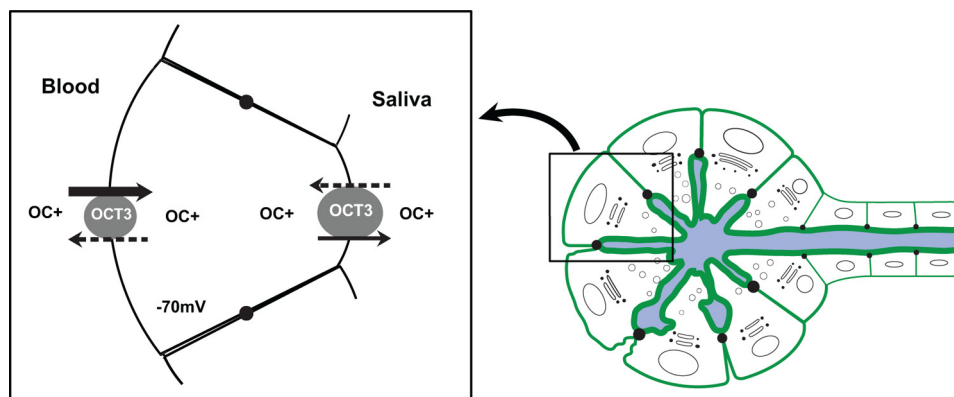
FIGURE 7. **Model proposed for OCT3-mediated organic cation (*OC+*) transport in salivary gland epithelial cells.** OCT3 on the basolateral membrane of epithelial cells mediates metformin uptake from the blood into the cells. Once metformin is highly concentrated inside the cells, OCT3 on the apical membrane facilitates efflux of metformin into the saliva. The *solid arrows* indicate the preferred direction of metformin transport when drug concentrations in the systemic circulation are high. The *dashed arrows* indicate the possible transport direction when systemic drug concentrations decline.

porters and their role in drug accumulation and secretion in salivary gland epithelial cells.

The primary function of salivary glands is to secrete saliva, which plays an important role in oral health, nutrient digestion, and immunity to microbial infection (30). About 0.75–1.5 liters of salivary fluid are secreted each day in healthy adults. Dysfunction of the salivary glands can lead to xerostomia, an uncomfortable and potentially harmful effect caused by medication use, radiation therapy, and autoimmune diseases (30, 31). Although xerostomia has many origins (3), excessive accumulation of foreign chemicals in salivary glands may lead to tissue toxicity and dysfunction of the salivary glands. Our *in vivo* studies revealed that Oct3-mediated active uptake can lead to very high metformin accumulation in salivary glands (Fig. 6). The overall tissue exposure to metformin in salivary glands is as high as that in the kidney, the major organ responsible for metformin elimination (Table 3). Oct3-mediated accumulation may thus intensify drug toxicity in salivary gland epithelial cells. Indeed, necrosis and inflammation of the salivary glands have been reported in rats chronically dosed with metformin (32). As OCT3/Oct3 is a polyspecific transporter, it can also transport other circulating drugs or toxins into salivary glands. Therefore, it is possible that Oct3-mediated or other carrier-mediated drug accumulation may interfere with the normal secretory function of salivary glands, contributing to hyposalivation and xerostomia.

In addition to salivary glands, there was a 33–34% reduction in metformin levels in the hearts of both male and female $Oct3^{-/-}$ mice (Table 3). Male $Oct3^{-/-}$ mice also showed significant changes in metformin exposure in skeletal muscle and liver. Furthermore, both male and female $Oct3^{-/-}$ mice exhibited altered plasma metformin kinetics, with a higher C_{max} and higher drug exposure during the first 60 min after drug administration. These pharmacokinetic changes in $Oct3^{-/-}$ mice most likely reflect a reduced distribution of metformin to peripheral tissues due to ablation of Oct3-mediated metformin uptake in many tissues, including, but not limited to, salivary glands and heart. Metformin is a first-line type 2 diabetes drug that acts as an insulin sensitizer and lowers blood glucose levels by suppressing hepatic gluconeogenesis, reducing intestinal glucose absorption, and stimulating glucose uptake and utiliza-

tion in skeletal muscle and adipose tissues (10, 11). Previous studies have suggested that hepatic and renal organic cation transporters (OCT1, OCT2, and MATE1) play an important role in influencing metformin pharmacokinetics and pharmacodynamics *in vivo* (10, 11). Genetic polymorphism of these transporters has been suggested to contribute to variable pharmacological response to metformin (10). Here, we showed that Oct3 impacts metformin pharmacokinetics *in vivo* and influences metformin distribution to tissue sites. Therefore, Oct3 could represent an additional genetic determinant of metformin disposition and action *in vivo*.

In summary, we demonstrated that OCT3 is responsible for metformin accumulation and secretion in salivary glands. Our study uncovered a novel carrier-mediated pathway for drug secretion into saliva and shed new light on the molecular mechanisms underlying drug-induced taste disorders. In addition, our study identified an important factor that could influence *in vivo* disposition of a mainstay drug used in the treatment of type 2 diabetes.

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