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Ability of sat-1 to transport sulfate, bicarbonate, or oxalate under physiological conditions

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Krick W, Schnedler N, Burckhardt G, Burckhardt BC. Ability of sat-1 to transport sulfate, bicarbonate, or oxalate under physiological conditions. *Am J Physiol Renal Physiol* 297: F145–F154, 2009. First published April 15, 2009; doi:10.1152/ajprenal.90401.2008.— Tubular reabsorption of sulfate is achieved by the sodium-dependent sulfate transporter, NaSi-1, located at the apical membrane, and the sulfate-anion exchanger, sat-1, located at the basolateral membrane. To delineate the physiological role of rat sat-1, [^{35}S]sulfate and [^{14}C]oxalate uptake into sat-1-expressing oocytes was determined under various experimental conditions. Influx of [^{35}S]sulfate was inhibited by bicarbonate, thiosulfate, sulfite, and oxalate, but not by sulfamate and sulfide, in a competitive manner with K_i values of 2.7 ± 1.3 mM, 101.7 ± 9.7 μM, 53.8 ± 10.9 μM, and 63.5 ± 38.7 μM, respectively. Vice versa, [^{14}C]oxalate uptake was inhibited by sulfate with a K_i of 85.9 ± 9.5 μM. The competitive type of inhibition indicates that these compounds are most likely substrates of sat-1. Physiological plasma bicarbonate concentrations (25 mM) reduced sulfate and oxalate uptake by more than 75%. Simultaneous application of sulfate, bicarbonate, and oxalate abolished sulfate as well as oxalate uptake. These data and electrophysiological studies using a two-electrode voltage-clamp device provide evidence that sat-1 preferentially works as an electroneutral sulfate-bicarbonate or oxalate-bicarbonate exchanger. In kidney proximal tubule cells, sat-1 likely completes sulfate reabsorption from the ultrafiltrate across the basolateral membrane in exchange for bicarbonate. In hepatocytes, oxalate extrusion is most probably mediated either by an exchange for sulfate or bicarbonate.

tubular reabsorption; basolateral membrane

SULFATE IS, AFTER chloride, bicarbonate, and phosphate, the fourth abundant anion in the blood, where its concentration is ~0.3–0.5 mM (1). Sulfate serves many biological functions. It is essential for the synthesis of various structural compounds such as glycosaminoglycans, the components of cartilage, or cerebroside sulfate, a constituent of myelin membranes. In the process of sulfate conjugation, various sulfotransferases catalyze the biotransformation and detoxification of xenobiotics, steroids, catecholamines, and bile acids for excretion (8). Since sulfotransferases are present in all cells and sulfate is a highly hydrophilic anion, cells have to be equipped with transport systems mediating the import of sulfate. The sulfate necessary for the above mentioned functions can be obtained either by intestinal absorption from the diet or by oxidation of the sulphur-containing amino acids methionine and cysteine (25).

In the kidneys, sulfate is freely filtered at the glomerulus and then undergoes, in healthy subjects, net reabsorption (1, 2). Using microperfusion studies in situ (4, 7, 16, 40), isolated perfused tubules (5), and membrane vesicles (9, 17, 21, 29, 30, 36), sulfate transport systems have been characterized at the luminal as well as at the basolateral membrane of proximal tubule cells. These studies revealed that sulfate uptake across the luminal membrane is mediated by a sodium-sulfate cotransport driven by the luminal *trans*-membrane sodium gradient (9, 21). The exit of sulfate across the basolateral membrane occurs by a sulfate-anion exchanger (4, 9, 17, 29, 36) showing affinities for oxalate and bicarbonate. In recent years, several transporters mediating sulfate and oxalate transport have been cloned. The transporter responsible for the luminal sodium-dependent entry step of sulfate was identified as NaSi-1 (slc13a1) (20, 23) and that is involved in the basolateral exit step as sat-1 (3, 12). Sat-1 is the first member of the SLC26 gene family which encodes anion exchangers transporting a wide variety of mono- and divalent anions (for review, see Refs. 25–27, 37).

In functional studies with sat-1 (slc26a1) expressed in *Xenopus laevis* oocytes for heterologous expression or Sf9 cells, sulfate uptake was inhibited by oxalate, bicarbonate, and thiosulfate (3, 12, 19, 24, 31, 32), confirming previous studies performed on basolateral membrane vesicles from rabbit kidney cortex (BLMV) (17) and rat kidney in situ (4, 7, 16, 40). Studies on rabbit BLMVs not only showed *cis* inhibition but also *trans*-stimulation of sulfate uptake by oxalate, bicarbonate, and thiosulfate and vice versa (17). Whereas transcripts of sat-1 were found in rat kidney (3), those for sat-1 were absent in rabbit kidney. Besides the effects of sulfate, bicarbonate, and thiosulfate on oxalate uptake, in rat kidney a sensitivity of oxalate uptake to probenecid and *p*-aminohippurate (PAH) was observed, leading to the assumption that more than one pathway for oxalate may exist in rat kidney (16).

The intention of the present study was, therefore, to test which of the above mentioned effects are mediated by sat-1. In addition, we wished to determine which of the transport modes may prevail at physiological concentrations of oxalate (39), sulfate (1), and bicarbonate (22), and therefore determined the affinities of rat sat-1 for various interacting anions.

MATERIALS AND METHODS

In vitro transcription of cRNA. Sat-1 cDNA from rat liver was used as a template for cRNA synthesis. Plasmids (pSport) were linearized with *Not*I and in vitro cRNA transcription was performed using the T7 mMessage mMaschine kit (Ambion, Austin, TX) according to the manufacturer's instructions. The resulting cRNA was resuspended in purified, RNAse-free water to a final concentration of 1 μg/μl.

Solutions. A standard oocyte Ringer solution (ORi) was used for oocyte preparation, storage, and for the experiments shown in Fig. 1*A* (gray column), Fig. 1*B* (gray column), and Fig. 2 ORi contained (in mM) 110 NaCl, 3 KCl, 2 $CaCl_2$, and 5 HEPES adjusted to pH 7.5 with

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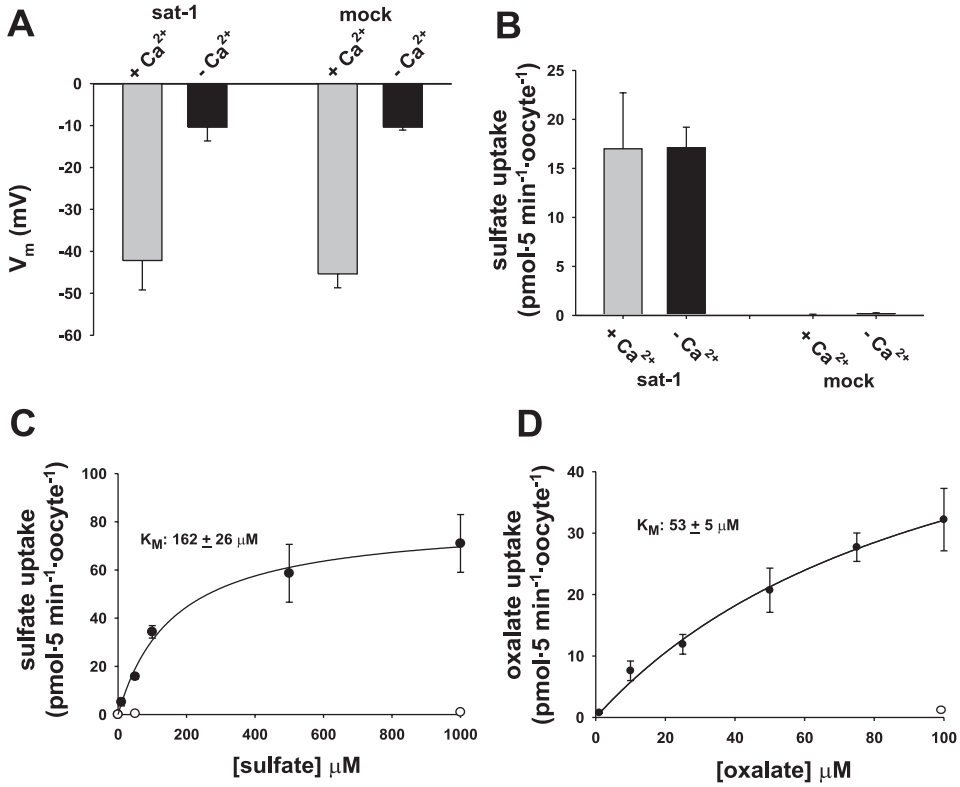

Fig. 1. Influence of calcium on sat-1. *A*: influence of calcium on membrane potential. Oocytes were either injected with water (mocks) or with 23 ng of rat sat-1 cRNA. After 3 days in culture, oocytes were first superfused with calcium-containing standard oocyte Ringer solution (ORi) and afterwards with nominal calcium-free ORi. Sat-1-expressing oocytes as well as mocks depolarized upon calcium removal. The data were collected after stabilization of the membrane potential (V_m). This occurred usually after 30 s of perfusion with the respective ORi. Data for sat-1 represent means ± SE of 14 oocytes from 8 donors and those for mocks of 7 oocytes from 5 donors. *B*: sulfate uptake in the presence and absence of calcium. [^{35}S]sulfate uptake over 5 min was measured in sat-1-expressing oocytes and mocks. Eight to 10 oocytes from 4 donors contributed to each data point. *C*: kinetics of sulfate transport. Oocytes were either not injected (○) or injected (●) with sat-1 cRNA. After 3 days in culture, 5-min [^{35}S]sulfate uptake was determined at room temperature in the presence of increasing sulfate concentrations. The data points represent means ± SE obtained from 8–10 oocytes from 3 donors. The K_m was obtained by fitting the curve by computer-based nonlinear regression analysis or by Eadie-Hofstee analysis with K_m values of 162 ± 26 and 156 ± 52 μM, respectively. *D*: kinetics of oxalate transport. Oocytes were either not injected (○) or injected (●) with sat-1 cRNA. After 3 days in culture, 5-min values of [^{14}C]oxalate uptake were determined at room temperature in the presence of increasing oxalate concentrations. The data points represent means ± SE obtained from 8–10 oocytes from 3 donors. The K_m was obtained by fitting the curve by computer-based nonlinear regression analysis or by Eadie-Hofstee analysis with K_m values of 53.5 ± 5.7 and 52.3 ± 5.1 μM, respectively.

Tris. After the independence of sulfate uptake on calcium was documented, all further sulfate and oxalate uptake and efflux studies (data shown in Fig. 1, *C* and *D*, and see Figs. 3 to 8) were performed under nominal calcium-free conditions, i.e., calcium was omitted from ORi (calcium-free ORi). Chloride-free solutions were obtained by substitution of NaCl and KCl by gluconate salts. The solutions containing 1 to 4 mM bicarbonate (Fig. 3*A*) were gassed with 1% CO_2-99% O_2. This leads to pH values of 6.9, 7.2, 7.4, and 7.5, respectively, at room temperature. Then, the pH of each solution was titrated to pH 7.5 by adding solutions of the respective bicarbonate concentration but not gassed with CO_2. By this procedure all solutions contain bicarbonate at pH 7.5 and varying CO_2 tensions. The 5 mM bicarbonate solution used for the experiments shown in Fig. 7*A* was also gassed with 1% CO_2-99% O_2. Due to the presence of HEPES/Tris, pH was 7.5. pH values of 8.5 and 9.5 were achieved by adding Tris. Solutions containing 25 mM bicarbonate at the expense of chloride (see Figs. 6*A* and 8, *B* and *C*) were titrated with HCl to achieve pH 7.5. pH values of all solutions containing bicarbonate were stable for more than 30 min when capped. In the experiments shown in Fig. 7*B*, pH was adjusted with Tris. The test anions (bicarbonate, thiosulfate, sulfamate, sulfite, sulfide, and oxalate) were purchased as sodium salts from Sigma (Taufkirchen, Germany).

Oocyte preparation and storage. Stage V and VI oocytes from *X. laevis* (Nasco, Fort Atkinson, WI) were separated by treatment with collagenase (Typ CLS II, Biochrom, Berlin, Germany) and maintained at 16–18°C in ORi. One day after removal from the frog, oocytes were injected either with 23 nl cRNA coding for sat-1 or an equivalent amount of water (mock) and maintained at 16–18°C in ORi supplemented with 50 μM gentamycin and 2.5 mM sodium pyruvate. After 3 days of incubation with daily medium changes, oocytes were used for tracer uptake and electrophysiological studies.

Transport experiments. Uptake of [^{35}S]sulfate (H_2SO_4; 1,200 Ci/mmol; Hartmann, Braunschweig, Germany) or [^{14}C]oxalate (100 mCi/mmol; Biotrend, Köln) in sat-1-expressing oocytes was assayed at room temperature. Dixon plots were performed by using either two sulfate concentrations (20 and 50 μM total sulfate with each 0.01 μM $^{35}SO_4^{2-}$ and increasing thiosulfate, sulfite, bicarbonate, and oxalate concentrations) or two oxalate concentrations (20 μM [^{14}C]oxalate and 50 μM [^{14}C]oxalate and increasing sulfate concentrations). *Trans*-stimulation experiments were performed by injection of either 23 nl of a 10 to 50 mM sodium sulfate, sodium oxalate, or sodium chloride solution into sat-1-expressing oocytes and mocks before the uptake of labeled sulfate or oxalate, respectively. After incubation in the respective solutions for 5 min, radioactivity was aspirated and the oocytes were washed twice in ice-cold ORi. Oocytes were dissolved by gently shaking for 2 h in 100 μl 1 N NaOH, neutralized with 100 μl 1 N HCl, and their ^{35}S or ^{14}C contents were determined by liquid scintillation counting (Tricarb 2900TR, Perkin Elmer, Rodgau, Germany). For sulfate efflux studies, sat-1-expressing oocytes and mocks were injected with 23 nl [^{35}S]sulfate. The oocytes were incubated for

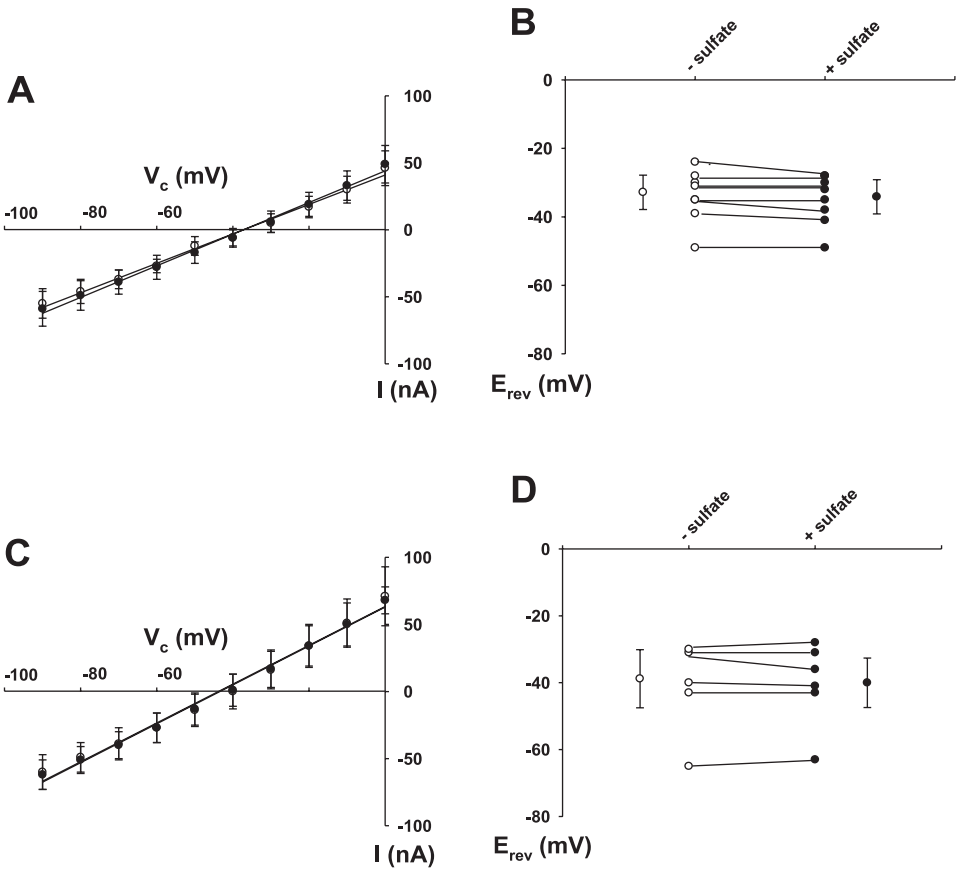

Fig. 2. Currents and reversal potentials (E_{rev}) in the absence and presence of sulfate in sat-1-expressing oocytes and mocks. Results shown in *A* and *B* were obtained on 8 sat-1-expressing oocytes from 6 frogs, whereas those in mocks (6 oocytes from 3 donors) are shown in *C* and *D*. Current-voltage (*I-V*) relationships (*A*, *C*) were measured in the absence (○) and presence of 0.5 mM sulfate (●). From the data presented in *A* and *C*, E_{rev} was calculated for sat-1-expressing oocytes (*B*) and mocks (*D*). Beside the data for the single experiments are the mean values at the *left* and *right* border.

30 min in calcium-free ORi containing increasing oxalate and bicarbonate concentrations at constant pH 7.5. At the end of the experiment, the ^{35}S content of each oocyte was assayed and expressed in percent of the injected amount which was set to 100%. Efflux experiments were performed with five oocytes for each experimental group. For every experimental condition, mocks were treated in a similar manner to serve as controls.

Two-electrode voltage-clamp studies. Three days after injection of cRNA or an equivalent amount of ORi, voltage recordings were performed in ORi in the presence and absence of calcium using a two-electrode voltage-clamp device (Warner OC725, Hamden, CT) in the current clamp mode (Fig. 1*A*). Steady-state currents were obtained during 5-s voltage pulses from −60 mV to potentials between −90 and 0 mV in 10-mV steps in the presence and absence of sulfate (0.5 mM) in the voltage-clamp mode (Fig. 2). For all two-electrode voltage-clamp measurements, borosilicate glass microelectrodes (Biomedical Instruments, Zöllnitz, Germany) filled with 3 M KCl were used.

Statistics. Student's *t*-test was used to show statistically significant differences of sulfate or oxalate uptake in the absence and presence of putative substrates for sat-1. Statistical significance was set at $^*P < 0.01$. The K_m values for sulfate and oxalate and K_i values for inhibition of sulfate or oxalate uptake by increasing concentrations of inhibitors were determined by SigmaPlot software (Systat Software, Point Richmond, CA) and by Eadie-Hofstee analysis.

RESULTS

To avoid precipitation of oxalate in calcium-containing solutions, calcium had to be omitted from ORi. Calcium removal caused a depolarization of 32.5 ± 5.0 and 35.0 ± 4.0 mV in sat-1-expressing oocytes and mocks (Fig. 1*A*), respectively. Despite this depolarization, sulfate uptake was not different in calcium-containing and calcium-free ORi (Fig. 1*B*), indicating that sulfate uptake was neither dependent on calcium nor on membrane potential. Under nominal calcium-free conditions, sulfate uptake into sat-1-expressing oocytes was saturable (Fig. 1*C*, ●), whereas mocks did not show any significant sulfate uptake (Fig. 1*C*, ○). The K_m for sulfate obtained under nominal Ca^{2+}-free conditions was calculated by the method of least square fits and was verified by Eadie-Hofstee analysis. In three separate experiments with 8-10 oocytes for each concentration, the former analysis revealed a K_m of 162 ± 26 μM, and the latter one a K_m of 156 ± 52 μM. [^{14}C]oxalate uptake into sat-1-expressing oocytes also tended to saturate with a K_m of 53.5 ± 5.7 μM (Fig. 1*D*, ●). A similar K_m (52.3 ± 5.1 μM) was calculated by Eadie-Hofstee analysis. Mocks showed no oxalate uptake (Fig. 1*D*, ○).

To confirm the independence of sulfate transport of membrane potential within a larger potential range, current-voltage (*I-V*) relationships on sat-1-expressing oocytes (Fig. 2, *A* and *B*) and mocks (Fig. 2, *C* and *D*) were performed in the presence of calcium to obtain more stable holding currents. In the absence (Fig. 2*A*, ○) as well as in the presence of sulfate (Fig. 2*A*, ●), *I-V* relationships were linear within a potential range between −90 and 0 mV. During these series, the reversal potential (E_{rev}) did not change significantly [−33.8 ± 5.0 mV in the absence of sulfate, −35.1 ± 5.0 mV in the presence of 0.5 mM sulfate; 8 oocytes from 6 donors; not significant (N.S.)]. In the individual experiments (Fig. 2*B*), four of eight oocytes slightly hyperpolarized upon application of sulfate, and four others did not. Mocks also showed linear *I-V* relationships in the absence as well as in the presence of sulfate

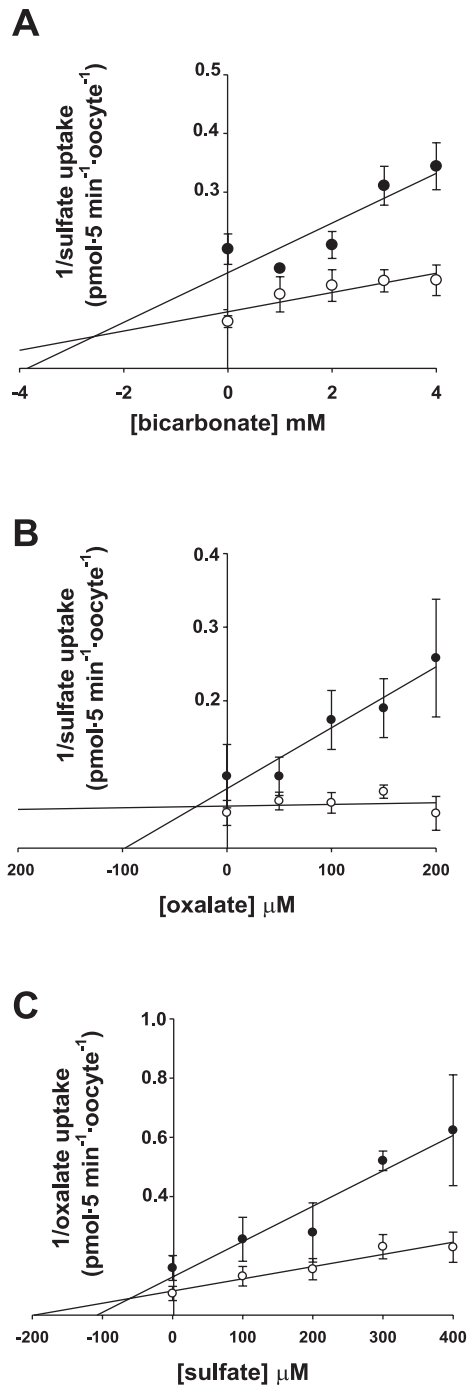

Fig. 3. Competitive inhibition of sulfate uptake by bicarbonate (*A*) and oxalate (*B*) and of oxalate uptake by sulfate (*C*). *A* and *B*: Dixon plot analysis revealed inhibition of sulfate uptake by bicarbonate and oxalate. K_i was determined by the concentration at the point of intersection of the straight lines evoked by the 2 sulfate concentrations. Inhibition of uptake was measured with either 20 (●) or 50 (○) μM total sulfate and increasing bicarbonate or oxalate concentrations at pH 7.5. The K_is determined in each 4 independent experiments for bicarbonate were 2.7 ± 1.3 mM and for oxalate were 63.5 ± 38.7 μM. *C*: Dixon plot analysis revealed a competitive inhibition of oxalate uptake with either 20 (●) or 50 (○) μM by sulfate with a K_i of 85.9 ± 9.5 μM as calculated from 3 experiments.

(Fig. 2*C*). As observed in six oocytes from three donors, E_{rev} changed from −39.1 ± 8.7 to −40.3 ± 7.4 mV (N.S.), and no systematic shift in E_{rev} was seen upon application of 0.5 mM sulfate (Fig. 2*D*). The lack of significant sulfate-induced changes in E_{rev} could be due to a too low expression of an electrogenic sat-1. However, the results presented in Fig. 1*B* (gray column) and those shown in Fig. 2 were obtained from the same batches of oocytes. [^{35}S]sulfate uptake into sat-1-expressing oocytes largely exceeded that into mocks, indicating a sufficiently high expression of sat-1. Hence, we conclude that sat-1 operates in an electroneutral fashion.

In recent studies, on BLMV from rabbit kidney (17) as well as from oocytes expressing sat-1 from various species (3, 19, 24, 31, 32, 42) and sat-1-transfected Sf9 cells (12), sulfate uptake was inhibited by oxalate (3, 12, 19, 24, 32, 42) and bicarbonate (31). To identify the type of inhibition of sulfate uptake by bicarbonate and oxalate, 20 and 50 μM sulfate and increasing bicarbonate and oxalate concentrations were used. Dixon plot analysis revealed a competitive type of inhibition for both anions with a K_i of 2.7 ± 1.3 mM for bicarbonate (Fig. 3*A*) and of 63.5 ± 38.7 μM for oxalate (Fig. 3*B*), respectively. Vice versa, *cis* inhibition of oxalate uptake by sulfate proved to be competitive with a K_i of 85.9 ± 9.5 μM (Fig. 3*C*).

The impact of other sulphur compounds, such as sulfamate, thiosulfate, sulfite, and sulfide (Fig. 4*A*), was also tested on sulfate uptake. Whereas sulfide and sulfamate showed no inhibition of sulfate uptake, thiosulfate (Fig. 4*B*) and sulfite (Fig. 4*C*) demonstrated competitive inhibition with K_i values of 101.7 ± 9.7 and 53.8 ± 10.9 μM, respectively.

As demonstrated in sulfate efflux studies with increasing oxalate concentrations in the bath, sat-1-expressing oocytes showed a reversed sulfate-oxalate exchange with a K_m for oxalate of 49 ± 25 μM (Fig. 5*A*). Vice versa, sulfate uptake was enhanced when oocytes were injected with oxalate (Fig. 5*B*), indicating exchange of intracellular oxalate with bath sulfate. Inversely, oxalate uptake (Fig. 5*C*) increased upon injection of sulfate. Before performing the experiments presented in Fig. 5, *B* and *C*, control experiments to exclude possible volume or osmotic alterations of the oocytes were done. Injection of either 23 nl of water or of a 50 mM NaCl solution did not change sulfate uptake. Sulfate uptake was virtually identical in these two groups with 14.0 ± 4.3 and 14.8 ± 3.8 pmol·5 min^{-1}·oocyte^{-1} (8-10 oocytes for each experimental conditions from 3 frogs), respectively, indicating that the enhanced uptake seen in the *trans*-stimulation experiments was an effect of the injected anion and not due to an increased volume or osmolarity.

Since sulfate uptake was inhibited by bicarbonate (Fig. 3*A*), sulfate efflux from sat-1-expressing oocytes and mocks into bicarbonate-free and bicarbonate-containing media was studied. In Fig. 6*A* the sulfate content measured in the oocytes upon incubation in bicarbonate-free (black columns) and bicarbonate-containing media (white columns) is shown. Whereas independent whether bicarbonate was present in the bath or not, no sulfate efflux was observed in mocks, sulfate efflux from sat-1-expressing oocytes was enhanced in a medium containing 25 mM bicarbonate as indicated by a lower sulfate content (Fig. 6*A*, white column). A part of sulfate efflux, however, was independent of bicarbonate. To investigate whether this bicarbonate-independent sulfate efflux was mediated by chloride, sulfate efflux in the presence and absence of chloride was

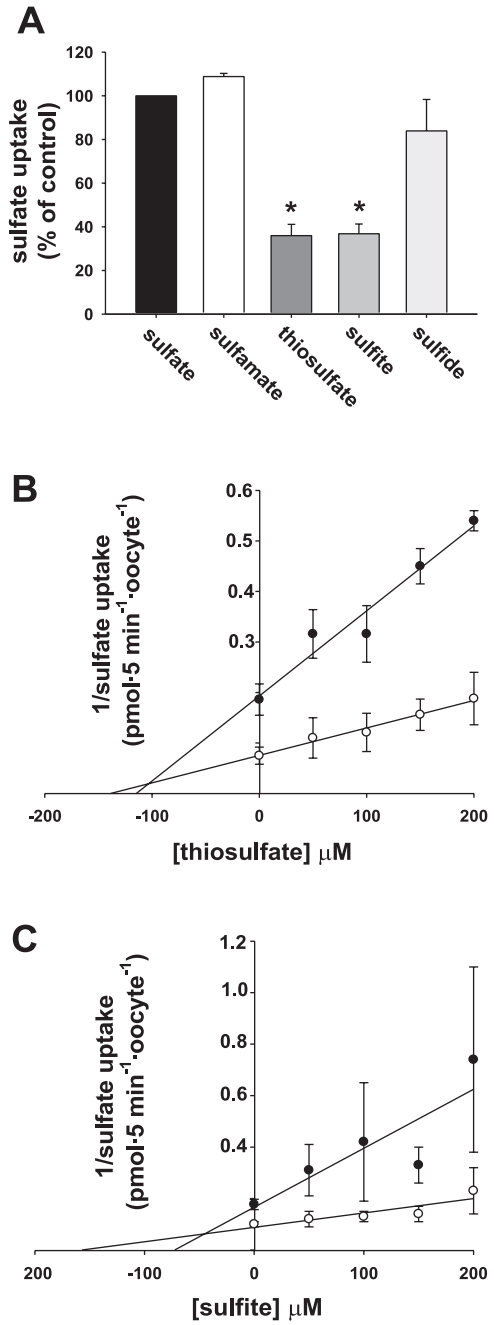

Fig. 4. Influence of anionic sulphur compounds on sulfate uptake. *A*: impact of sulfamate, sulphide, sulfite, and thiosulfate (each 1 mM) was tested on 20 μM sulfate uptake (3 experiments with each 8-11 oocytes, $*P < 0.01$ vs. control). Thiosulfate (3 experiments with 8-10 oocytes for every experimental condition; *B*) and sulfite (4 experiments with 8-11 oocytes for each experimental condition; *C*) showed competitive inhibition of sulfate uptake [20 (●) and 50 (○) μM sulfate].

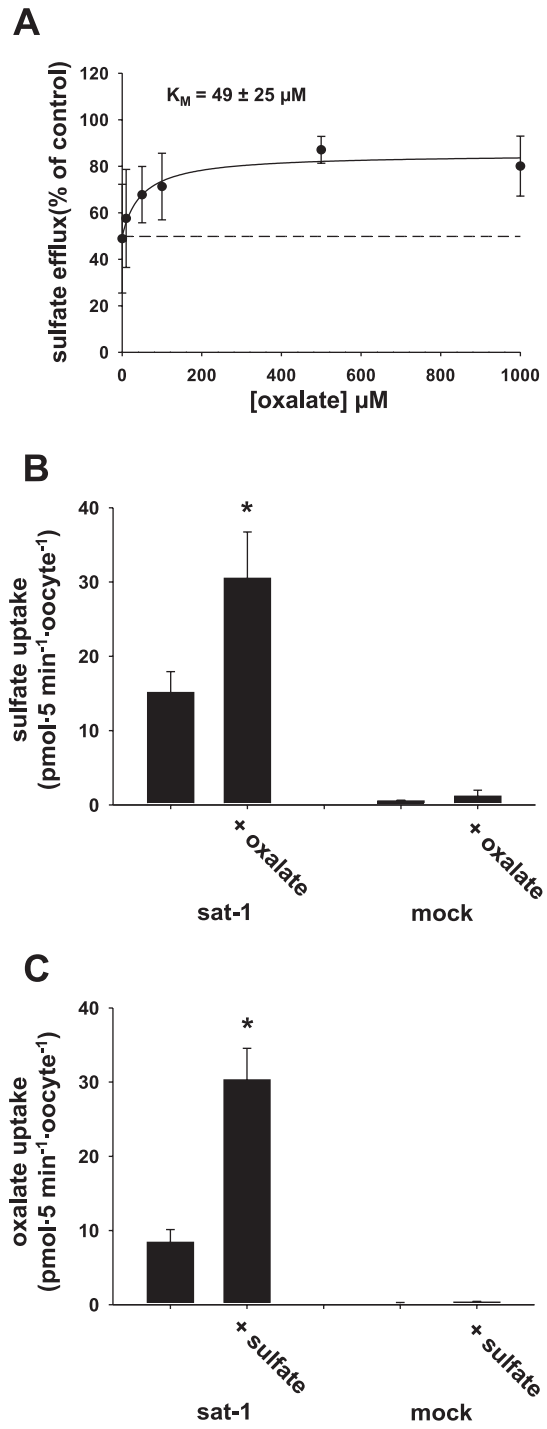

Fig. 5. Stimulation of sulfate efflux by oxalate and demonstration of sulfate-oxalate and oxalate-sulfate exchange. *A*: increasing oxalate concentrations in the bath stimulated sulfate efflux over 30 min as observed in 4 experiments with 5 oocytes per condition. The dashed line represents sulfate efflux independent of oxalate. Sulfate efflux in mocks was 3.0 ± 0.6% of the injected amount of sulfate at 1 mM oxalate (data not shown). In the *trans*-stimulation experiments either sulfate (*B*) or oxalate (*C*) uptake was measured upon injection of oxalate (23 nl of a 10 mM solution; *B*) and of sulfate (23 nl of a 25 mM solution; *B*) before the experiment. Data represent 8–10 oocytes under each experimental condition. Each experimental condition was statistically different from control by $*P < 0.01$.

studied (Fig. 6*B*). [^{35}S]sulfate efflux was enhanced in chloride-containing media as documented by the lower sulfate content within the oocytes bathed in chloride compared with chloride-free, gluconate-containing media.

In previous studies, uptake of sulfate by sat-1 had been shown as pH dependent (42). A one-unit change of bath pH from 7.5 to 8.5 and 9.5 reduced sulfate uptake (Fig. 7*A*, black columns). In Fig. 3, *A* and *B*, competitive inhibition of sulfate

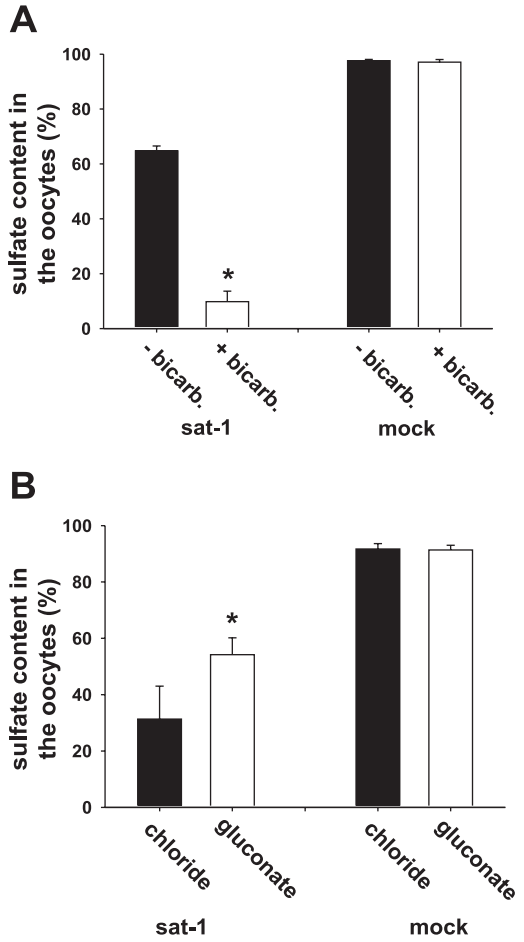

Fig. 6. Stimulation of sulfate efflux by bicarbonate and chloride. Sulfate efflux was measured over 30 min in media containing either no or 25 mM bicarbonate (*A*) or chloride or gluconate (*B*). The amount of injected [^{35}S]sulfate in the oocytes before the incubation in the different media was set to 100%. Data represent 5 oocytes for each experimental condition from 3 (*A*) or 4 (*B*) frogs. Whereas sulfate efflux from mocks was negligible under all experimental conditions, sulfate efflux of sat-1-expressing oocytes was larger in bicarbonate- (*A*) and in chloride-containing (*B*) than in bicarbonate- or chloride-free media with $*P < 0.01$.

uptake by bicarbonate and by sulfite was shown. To receive further information whether the mono- (HCO_3^-/HSO_3^-) or the divalent compound (CO_3^{2-}/SO_3^{2-}) was responsible for the inhibition, sulfate uptake in the absence and presence of bicarbonate (Fig. 7*A*) and sulfite (Fig. 7*B*) was studied at different extracellular pH values. At pH 7.5, application of 5 mM HCO_3^-/CO_3^{2-} reduced sulfate uptake to 16.7 ± 11.6% of its initial value (Fig. 7*A*). The percentage of inhibition decreased continuously at increasing pH, although the amount of carbonate increased from 0.2% at pH 7.5 to 15.9% at pH 9.5. Measuring inhibition of sulfate uptake by 1 mM HSO_3^-/SO_3^{2-} at pH 7.5 and pH 8.5 showed a larger inhibiton of sulfate uptake at pH 7.5 (Fig. 7*B*), although the amount of sulfite increased from 70% at pH 7.5 to 95% at pH 8.5.

Because the expression of sat-1 varied during the course of the study, we approximated V_{max} values of sulfate and oxalate within one batch of oocytes. In four experiments using saturating sulfate (1.5 mM) and oxalate (0.5 mM) concentrations, apparent V_{max} values of 36.6 ± 9.0 for sulfate and 22.0 ± 6.2 pmol·5 min^{-1}·oocyte^{-1} for oxalate were obtained, respectively (Fig. 8*A*). Subtraction of sulfate and oxalate uptake by mocks revealed uptakes of 33.4 ± 7.8 and 20.1 ± 5.9 pmol·5 min^{-1}·oocyte^{-1}, respectively. These values were not statistically different ($P > 0.1$; Fig. 8*A*, *inset*). To evaluate the physiological role of sat-1 in sulfate and oxalate transport, [^{35}S]sulfate and [^{14}C]oxalate uptake was measured in the presence of the plasma concentrations for bicarbonate (25 mM), sulfate (0.3 mM), and oxalate (0.02 mM). Sulfate uptake was inhibited by 78.3 ± 10.6% in the presence of bicarbonate (Fig. 7*B*), and oxalate had no marked effect on sulfate uptake. The simultaneous presence of bicarbonate and oxalate led to a further decline in sulfate uptake to 8.0 ± 4.0% of its initial value. Oxalate uptake was inhibited by 70.8 ± 2.8 and 94.9 ± 1.7% by physiological concentrations of sulfate and bicarbonate (Fig. 8*C*), respectively. Simultaneous application of sulfate and bicarbonate nearly abolished oxalate uptake. Under these conditions, oxalate uptake was only 2.6 ± 0.6% of that measured in the absence of sulfate and bicarbonate.

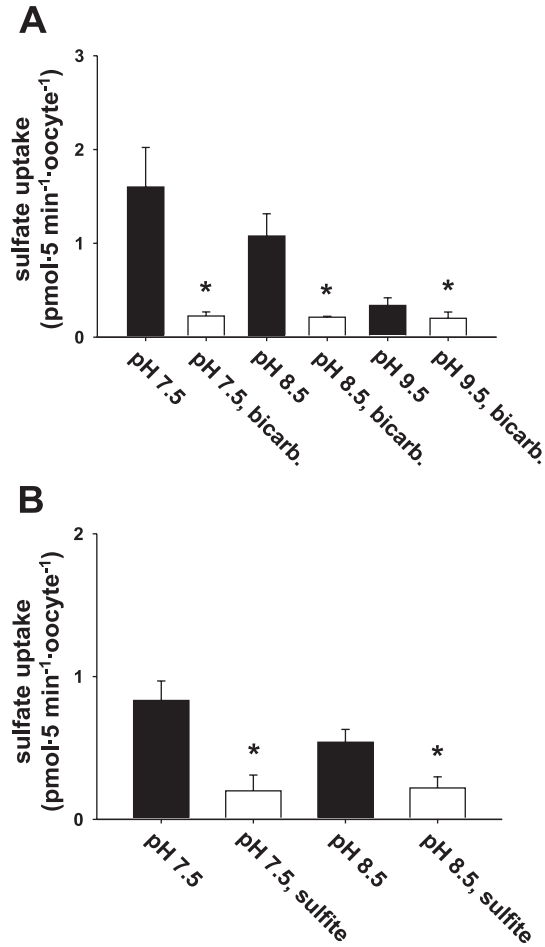

Fig. 7. pH dependence of sulfate uptake. *A*: sulfate uptake (20 μM) was measured at pH 7.5, 8.5, and 9.5 in the absence (filled bars) and presence of 5 mM bicarbonate (open bars) and *B*: at pH 7.5 and 8.5 in the absence (filled bars) and presence of 1 mM sulfite (open bars). Data shown in *A* represent 4 and those shown in *B* 3 experiments with 8-10 oocytes for each experimental condition. The uptake of sulfate in mocks under these conditions was low (ranging from 0.045 ± 0.012 to 0.075 ± 0.024 pmol·5 min^{-1}·oocyte^{-1}) and subtracted from the individual values.

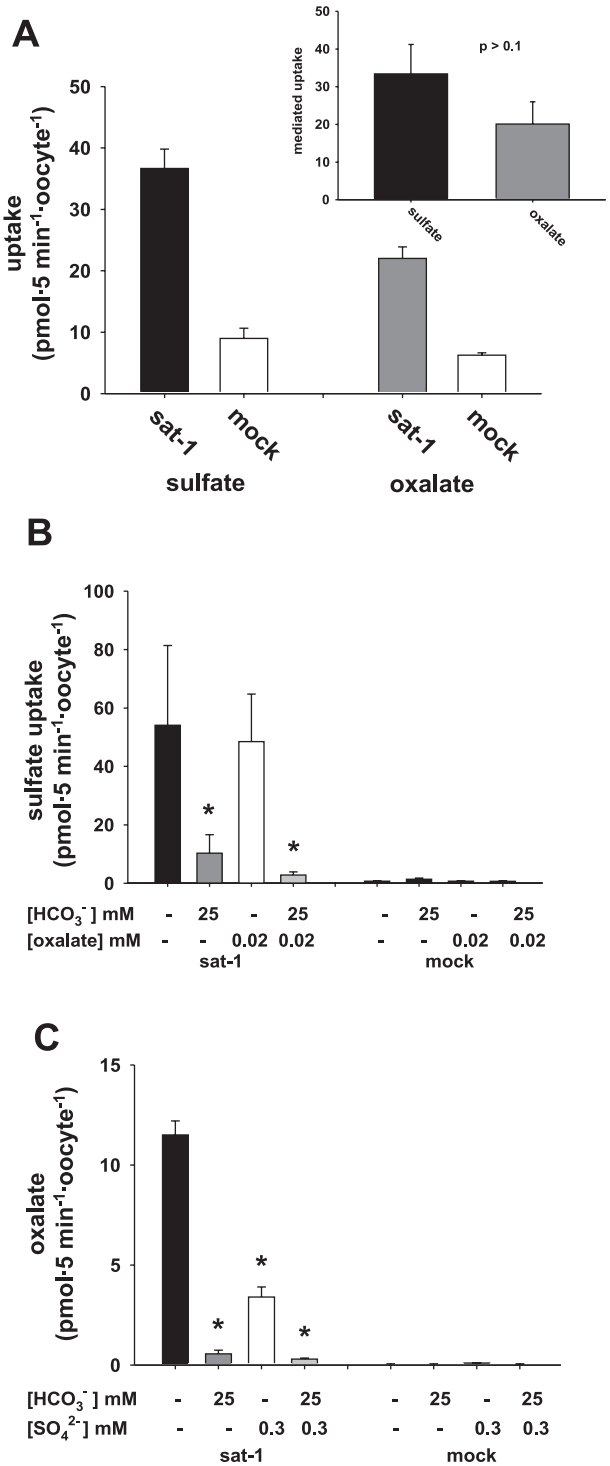

Fig. 8. Comparison of sulfate and oxalate uptake and evaluation of the physiological role of oxalate. *A*: sulfate or oxalate uptake was measured simultaneously in the same 3 batches of oocytes at saturating sulfate (1.5 mM) and oxalate (0.5 mM) concentrations. *B*: sulfate uptake was measured under physiological concentrations of sulfate (0.3 mM), bicarbonate (25 mM), and oxalate (0.02 mM). *C*: oxalate uptake was measured in the dependence of physiological concentrations of oxalate (0.02 mM), sulfate (0.3 mM), and bicarbonate (25 mM). The data shown in *B* and *C* were obtained in 3 different batches of oocytes with each 8–10 oocytes per experimental condition (*$P <$ 0.01 vs. sole sulfate or oxalate uptake, respectively).

DISCUSSION

In humans and rodents, sulfate absorption takes place in the small intestine. Following distribution in the circulation, sulfate is freely filtered at the glomerulus and actively reabsorbed in the proximal tubule. The electrochemical potential difference for sodium across the brush-border membrane provides the driving force for the intracellular accumulation of sulfate by a sodium sulfate cotransporter, NaSi-1 (20, 21). Sat-1, a sulfate-anion exchanger located at the basolateral membrane (3, 12), facilitates the exit of sulfate out of the cell along its electrochemical gradient.

To avoid the precipitation of calcium oxalate, all experiments, except those shown in Fig. 1*A* (gray columns), Fig. 1*B* (gray columns), and Fig. 2, were performed under nominal calcium-free conditions. Neither sulfate uptake by sat-1 nor the K_m for sulfate was dependent on calcium. The K_m determined in the nominal absence of calcium was 0.162 mM and was similar to that determined in the presence of calcium in earlier studies (3). Removal of calcium caused a cell membrane depolarization of 30 mV. Despite this depolarization, sulfate uptake was unchanged, indicating that sulfate uptake was not potential dependent. Studies with a larger potential range supported electroneutral sulfate-anion exchange, since the shift in reversal potential upon application of sulfate was not different in sat-1-expressing oocytes and mocks. Presently, it is not clear which intracellular anion serves as the exchange partner. Due to the observation that sulfate uptake was larger in the presence of chloride, it was suggested that intracellular chloride served as an exchange partner (19, 32, 42). This assumption, however, was disproven by the finding that intracellular chloride concentration in oocytes expressing rat sat-1 did not change upon application of sulfate (42). Oocytes expressing sat-1 from mouse or human, however, did show changes in intracellular chloride concentrations upon application of sulfate (19, 32). These observations suggest species differences with respect to chloride. To reevaluate the chloride dependence, sulfate efflux in either chloride-containing or chloride-free media was measured. Sulfate efflux from sat-1-expressing oocytes but not from mocks (Fig. 6*B*) was enhanced by the presence of chloride, indicating that at least a part of sulfate efflux may occur by sulfate-chloride exchange. This part may be so small that it could not be detected by Xie et al. (42) due to the low specific activity of radiolabeled chloride. Alternatively, chloride may exert an allosteric effect, thereby facilitating sulfate binding and/or sulfate transport.

Sulfate uptake was significantly inhibited by oxalate in a competitive manner with a K_i of 63.5 μM, a value close to the oxalate concentration detected in the serum of patients with hyperoxaluria or end-stage renal disease (38). Uptake of oxalate showed saturation kinetics with a K_m of 53.5 μM, a value close to that obtained for the K_i for competitive inhibition of sulfate uptake by oxalate (63.5 μM), indicating an interaction at the same site of sat-1. Sulfate uptake increased when sat-1-expressing oocytes were injected with oxalate and, vice versa, oxalate uptake increased when sulfate was injected (Fig. 5, *B* and *C*). In addition, sulfate efflux increased when oxalate was present in the bath (Fig. 5*A*). All these observations are compatible with a bidirectional sulfate-oxalate exchange shown earlier with BLMV from rabbit kidney (17).

Bicarbonate is another anion showing competitive inhibition of sulfate uptake with a K_i of 2.7 mM (Fig. 3*A*). Sulfate efflux increased when bicarbonate was present in the bath (Fig. 6*A*). These results and earlier findings that sulfate uptake was inhibited by the carbonic anhydrase inhibitor acetazolamide (31) and stimulated by an acidic extracellular pH (31, 42) furthermore support the presence of sulfate-bicarbonate exchange by sat-1. Changing extracellular pH from physiological to more alkaline values reduced the relative inhibition of sulfate uptake by HCO_3^-/CO_3^{2-} as well as by HSO_3^-/SO_3^{2-}, indicating that the interactions of carbonate or sulfite are weak in contrast to bicarbonate or hydrogensulfite.

Thiosulfate ($S_2O_3^{2-}$) and sulfite (SO_3^{2-}) are intermediates in sulfide oxidation to sulfate or are produced by the thiol catabolism of cysteine (11, 38). Degradation of sulfide occurs in mitochondria as well as in the cytosol in several organs including liver, kidneys, and brain. Thiosulfate is freely filtered at the glomerulus, but unlike sulfate, not reabsorbed in the proximal tubule. It is, however, secreted into the lumen of the proximal tubule and excreted by the urine (2, 7). Data on the excretion of sulfite are not available. Thiosulfate and sulfite showed competitive inhibition of sulfate uptake with nearly identical K_i values (101.7 ± 9.7 and 53.8 μM), indicating a high affinity of sat-1 for these anions. In individuals homozygous for sulfite oxidase deficiency, sulfite and thiosulfate accumulate in the body. Sulfite oxidase deficiency is a disease that primarily affects the central nervous system. The neuropathogenesis of sulfite oxidase deficiency remains unclear. It is speculated that the insulating capacity and stability of myelin are impaired due to undersulfated cerebrosides (38). In situ hybridization showed that in the mouse brain, sat-1 mRNA is localized in the hippocampus and the cerebellum (18).

The question which of the anions is transported under physiological concentrations of sulfate, bicarbonate, and oxalate has not been solved so far. Recent determinations of the mean oxalate/creatinine clearance in human revealed values between 0.79 to 2.0, indicating reabsorption as well as secretion of oxalate (13, 14, 41). Plasma oxalate concentrations of healthy subjects are usually in the range of 1–5 μM (13, 14, 39). Using the K_m determined for oxalate and the Michaelis-Menten equation, sat-1 is theoretically 1.8–8.6% occupied by oxalate. Under this condition, sat-1-mediated net secretion may not occur. In patients with hyperoxaluria in which plasma oxalate concentrations reach values up to 100 μM (39) occupancy may increase to 65.3% and oxalate is taken up into the cell. Since sat-1 cooperates with CFEX (slc26a6), an oxalate-anion exchanger located at the luminal membrane, oxalate secretion may occur and may contribute to the formation of calcium oxalate stones.

At least at physiological concentrations the major substrates of sat-1 seem to be sulfate and bicarbonate, but not oxalate. Taking the physiological plasma concentration of bicarbonate (22), the extracellular binding site of sat-1 would be saturated by ~90% with bicarbonate alone. The intracellular concentrations of sulfate and oxalate are not known. For sulfate, the

Table 1. *Kinetic parameters of sulfate-bicarbonate-oxalate exchange*

Compound	K_m, μM*	K_m, μM†	K_i, μM Sulfate Uptake‡	K_i, μM Oxalate Uptake‡
Sulfate	162±26[a]			85.9±9.5
	156±52[b]			
Oxalate	53.5±5.7[a]			
	52.3±5.1[b]	49.0±25.0	63.5±38.7	
Bicarbonate			2,700±1,300	
Thiosulfate			101.7±9.7	
Sulfite			53.8±10.9	

Data for sulfate and oxalate represent means ± SE obtained from 8-10 oocytes from 3 donors. *Determined by influx studies. Either 20 μM [^{35}S]sulfate or [^{14}C]oxalate and increasing unlabeled sulfate or oxalate concentrations were used to reach the final concentrations indicated in Fig. 1, *C* and *D*, respectively. Data were obtained either by SigmaPlot[a] calculations or by Eadie-Hofstee[b] analysis. The corresponding graphs were shown in Fig. 1, *C* and *D*. †Determined by sulfate efflux. Twenty-three nanoliters of [^{35}S]sulfate were injected into sat-1-expressing oocytes and sulfate efflux was followed for 30 min at increasing unlabeled oxalate concentrations (10, 25, 50, 100, 500, and 1,000 μM). Since sulfate was exchanged against oxalate, the amount of sulfate within the oocytes at different oxalate concentrations in the bath was used to calculate the K_m. Data were obtained from 4 different frogs with each 5 oocytes per experimental condition. ‡Determined by *cis* inhibition experiments and calculated by Dixon plots as shown in Figs. 3 and 4.

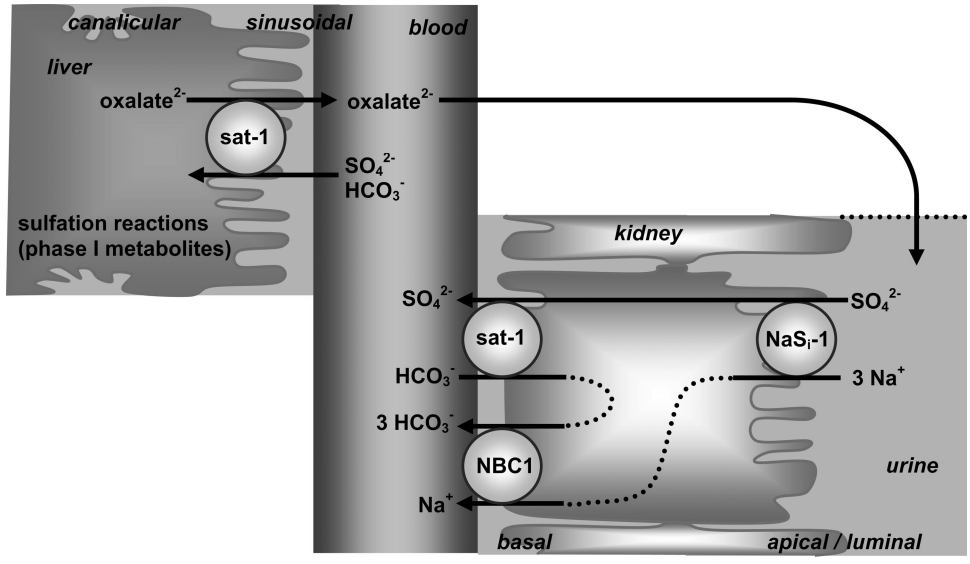

Fig. 9. Schematic summary for the physiological role of sat-1 in hepatocytes and proximal tubular cells. For explanation, see text. Sat-1, sulfate anion exchanger cloned by Ref. 3 and localized by Ref. 12; NaSi1, sodium sulfate cotransporter 1 cloned by Ref. 23 and localized by Ref. 20; NBC1, sodium bicarbonate cotransporter 1 cloned by Ref. 33 and localized by Ref. 35.

intracellular concentration is likely to be higher than the extracellular one due to sodium-driven sulfate uptake at the luminal membrane (21, 23). The intracellular bicarbonate concentration is lower than the extracellular one (43). Hence, we propose that in proximal tubular cells the intracellular site of sat-1 preferably binds sulfate, whereas the extracellular site binds bicarbonate, leading to sulfate exit into the blood and bicarbonate uptake into the cell. The bicarbonate taken up by sat-1 must be extruded by the sodium-bicarbonate-carbonate cotransporter NBC1 (slc4a4) (33, 34). Since [^{35}S]sulfate uptake is independent of membrane potential (Figs. 1*A* and 2) and carbonate (CO_3^{2-}) does not seem to be a substrate of sat-1, translocation of two HCO_3^- in exchange with SO_4^{2-} would be compatible with an electroneutral exchange mode. Furthermore, the observation that application of sulfate to sat-1-expressing oocytes preincubated for 4 h in a bicarbonate-containing solution did not evoke sulfate-mediated currents (data not shown) supports electroneutral sulfate-bicarbonate exchange. Such an electroneutral mode may also be present in oxalate^{2-}:SO_4^{2-} exchange or in 2 Cl^-:SO_4^{2-} exchange, as favored by Markovich et al. (19, 32). However, whether chloride (Fig. 6*B*) is transported or acts allosterically on rat sat-1 cannot be answered at this time.

In hepatocytes, sat-1 is located in the sinusoidal membrane. The intracellular concentration of oxalate in hepatocytes is assumed to be high due to its production from glycine and hydroxyproline (6, 15, 28) and oxalate should occupy the intracellular binding site of sat-1. Oxalate is extruded in exchange for bicarbonate or sulfate. This mechanism contributes either to pH homeostasis or supplies the hepatocytes with sulfate for sulfation reactions. A similar mechanism has recently been described by Hugentobler and Meier (10) in sinusoidal membrane vesicles. In Fig. 9 a model describing these pathways for sulfate, oxalate, and bicarbonate in hepatocytes and kidney proximal tubular cells is provided. Oxalate is synthesized in the liver and extruded into the circulation by sat-1. In the absence of hyperoxaluria, renal filtration of oxalate is sufficient to maintain low blood oxalate concentrations. Sulfate is also freely filtered at the glomerulus and absorbed in kidney proximal tubule cells by NaSi-1. At the basolateral membrane intracellular sulfate exchanges against bicarbonate. To avoid intracellular alkalinization by this process, bicarbonate has to be extruded by NBC1. From the peritubular capillaries, sulfate is transported via blood to liver hepatocytes where sulfate is taken up by sat-1 to facilitate sulfation reactions. Alternatively, bicarbonate can be taken up by sat-1 in exchange for oxalate. All modes of bidirectional sulfate-oxalate, sulfate-bicarbonate, and oxalate-bicarbonate exchange have been demonstrated in this paper.

In summary, we showed that rat sat-1 interacts with sulfate, thiosulfate, bicarbonate, and oxalate, which is in agreement with earlier studies on rat and rabbit renal membrane vesicles and oocytes expressing sat-1 from various species. In addition, we showed that sulfate uptake was inhibited by sulfite but not by sulfide and sulfamate. Based on the affinities for oxalate, sulfate, and bicarbonate (Table 1), we propose that, under physiological conditions, sat-1 exchanges intracellular sulfate for extracellular bicarbonate that is recycled by NBC1. Sat-1 may significantly contribute to oxalate excretion only in hyperoxalemic patients. Given the functions in proximal tubule cells and in hepatocytes, a sat-1-null phenotype would probably lose sulfate with the urine and accumulate oxalate in hepatocytes.

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