

Iodide Transport in Lactating Rat Mammary Tissue via a Pathway Independent from the Na^+/I^- Cotransporter: Evidence for Sulfate/Iodide Exchange

D. B. Shennan

Hannah Research Institute, Ayr, Scotland, United Kingdom KA6 5HL

Received January 5, 2001

Although it is beyond doubt that mammary cells accumulate iodide via a Na^+ -dependent transport mechanism, it is not clear if this is the only pathway for iodide transport in mammary tissue. In view of this, experiments were designed to test for the presence of an anion-exchange pathway which could mediate the transport of iodide into mammary cells; thus, the effect of external iodide on sulfate efflux from rat mammary tissue has been investigated. Iodide *trans*-stimulated sulfate efflux from mammary tissue explants in a dose-dependent manner: 0.1, 1.0 and 10.0 mM iodide stimulated the fractional release of iodide by 56 ± 2.2 , 166.5 ± 17.5 , and $302.9 \pm 29.8\%$, respectively. The stimulation of sulfate efflux by external iodide was completely inhibited by DIDS (4,4'-diisothiocyanatostilbene 2,2'-disulfonic acid). Perchlorate (1 mM) also *trans*-stimulated sulfate efflux in a manner that was inhibited by DIDS. Furthermore, iodide *trans*-accelerated sulfate efflux from rat mammary acini via a DIDS-sensitive mechanism. The results are consistent with the presence of a DIDS-sensitive anion-exchange mechanism which can accept iodide as a substrate. It appears that the iodide-sulfate exchange mechanism is independent from the sodium-dependent iodide transporter given that sulfate is not a substrate of the latter system. The iodide-sulfate exchanger may operate in parallel with the sodium-dependent iodide transporter to mediate iodide uptake into mammary cells. © 2001 Academic Press

Key Words: iodide transport; mammary; anion-exchange.

Iodide is concentrated in both milk and mammary tissue with respect to plasma (1, 2). The transport of iodide by the mammary gland was investigated relatively early because of the possible contamination of milk supplies by radioactive iodide (3). The importance of thoroughly understanding iodide transport by the mammary gland was highlighted by the many cases of

human thyroid cancer that followed the Chernobyl nuclear accident (4, 5): radioactive iodide entering the food chain via milk and dairy products could have been a major contributing factor. The study of mammary gland iodide transport has been given new impetus by the recent finding that a mammary gland iodide transporter expressed in lactating mammary cells (but not in non-lactating breast tissue) is also present in breast cancer. It has been suggested that radioactive iodide uptake could be used to diagnose and treat breast cancer (6).

Iodide can enter mammary cells via a Na^+ -dependent process which can be inhibited by anions such as perchlorate and thiocyanate (e.g., see 7, 8). In addition, the mammary tissue Na^+/I^- cotransporter appears to be regulated by prolactin (8–10). It appears, therefore, that the properties of iodide transport by the mammary gland are similar to those of the thyroid gland with respect to ion dependency and inhibition by anions. In this connection, mRNA encoding a Na^+ -dependent transporter (NIS) similar to that found in the thyroid gland (11) has been identified in the mammary gland (12).

Although it is beyond doubt that the Na^+ -dependent iodide transport system is central to iodide accumulation is not clear if this is the only pathway for iodide transport in mammary tissue. In this connection there are several lines of evidence to suggest that there may be more than one pathway for iodide uptake by the mammary gland. First, it appears that cultured mouse mammary tissue is able to concentrate radiolabeled iodide in the absence of extracellular sodium (8). Second, the mammary gland of pregnant rats can concentrate iodide *in vivo* even though the sodium-dependent iodide transport protein could not be detected in mammary tissue isolated from pregnant rats (13). Third, there is preliminary evidence that iodide is able to *trans*-stimulate sulfate efflux from rat mammary tissue (14).

Given the importance of iodide uptake by the mammary gland it is imperative that all the pathways for

iodide transport in mammary tissue are elucidated. In view of this we have studied the interaction between iodide and sulfate transport in lactating rat mammary tissue as a means of examining the possibility that iodide uptake into mammary cells can be mediated via an anion-exchange mechanism.

MATERIALS AND METHODS

Animals and preparation of tissue. Lactating Wistar rats 10–15 days postpartum and suckling 8–10 pups were used in this study. The animals were maintained on a 12 h light:12 h dark cycle and allowed free access to water and chow. Animals were killed by cervical dislocation. Mammary explants (each 4–8 mg wet weight) were prepared as described by Shennan *et al.* (15). Mammary acini were prepared as follows. 5 g of mammary tissue was suspended in 10 ml of a buffer containing (mM) 135 NaCl, 5 KCl, 2 CaCl₂, 1 MgSO₄, 10 glucose and 20 Tris–Mops, pH 7.4, and finely chopped. The tissue was then strained and added to 30 ml of a buffer similar in composition to that just described except that it also contained 1.5 g Ficoll 400, 600 mg bovine serum albumin and 30 mg of collagenase. The tissue was incubated at 37°C for 40 min in a shaking water bath. Following this the digest was strained and the eluent centrifuged at 550g for approximately 15 s. The pellet was washed (×3) by centrifugation and resuspension.

The efflux of sulfate, using ³⁵SO₄²⁻ as tracer (Amersham plc UK), was measured according to the method of Shennan *et al.* (15). Mammary tissue explants were loaded with radiolabeled sulfate by a 1 h incubation at 20°C in a medium containing (mM) 145 Na gluconate, 10 glucose and 10 Tris–Mops, pH 7.4, plus 10 μCi/ml ³⁵SO₄²⁻. After the loading period, the tissue explants were passed through a series of tubes containing 2 ml of non-radioactive solutions (see figure legends for precise details of composition) maintained at 20°C at 2 min intervals. At the end of the wash-out period the explants were placed in 4 ml of distilled water for at least 16 h in order to leach out the isotope remaining in the tissue. The fractional efflux was calculated for each collection period by calculating the ratio of the radioactivity lost from the tissue to the amount of radioactivity associated with the tissue at the start of each collection period. The concentration of iodide in the buffers was routinely checked using an iodide-sensitive electrode (Russel pH Limited Model 94-4530) in conjunction with a Jenway 3340 ion meter.

Measurement of SO₄²⁻ from mammary acini. Mammary acini, prepared as described above were suspended in a solution containing (mM) 145 Na gluconate, 10 glucose and 10 Tris–Mops, pH 7.4 plus 10 μCi/ml of ³⁵SO₄²⁻ for approximately 25 min at 20°C. Following the loading period the acini were washed (×3) in rapid succession by centrifugation and resuspension with a buffer containing (mM) 145 Na gluconate 10 glucose and 10 Tris–Mops, pH 7.4, to remove extracellular radiolabeled sulfate. After the final wash the acini were suspended in 1 ml of buffer (see figure legends for details of the composition). After 3 min, the acini were centrifuged for 5 s. The supernatant was removed (and prepared for counting) and the pellet was resuspended in 1 ml of incubation buffer. This process was repeated throughout the time course at 3 min intervals. The amount of radioactivity associated with the acini at the end of the efflux period was determined by adding 1 ml of distilled water to the pellet. After 2 h the suspension was centrifuged and 0.5 ml of the supernatant was prepared for counting. The fractional efflux of radiolabeled sulfate was calculated for each period as described above for sulfate efflux from mammary tissue explants.

Statistical analysis. Differences were assessed by Student's paired or unpaired *t*-test as appropriate and were considered significant when *P* < 0.05.

RESULTS

The Effect of Iodide on Sulfate Efflux from Rat Mammary Tissue Explants

The first step in the investigation was to test the effect of external iodide on the fractional release of radiolabeled sulfate from mammary tissue explants. Sulfate efflux was initially measured into a buffer containing gluconate as the principal anion. Gluconate was chosen as it is a relatively impermeant monovalent anion. Preliminary experiments revealed that sulfate efflux from rat mammary explants could be described by at least two components (results not shown). The fast component, which was extracellular in origin, was minimal by 30 min. Therefore, sulfate efflux was measured from explants incubated in a gluconate buffer (iodide-free) for 40 min before the tissue was transferred into a buffer containing iodide. It is evident from Fig. 1a that external iodide *trans*-stimulated sulfate efflux in a dose-dependent manner. Iodide at 0.1, 1.0 and 10 mM respectively increased the fractional efflux (basal-to-peak) by $0.0065 \pm 0.0006 \text{ min}^{-1}$ (\pm SE, *n* = 3, *P* < 0.01), $0.0185 \pm 0.0021 \text{ min}^{-1}$ (\pm SE, *n* = 9, *P* < 0.001) and $0.0344 \pm 0.0031 \text{ min}^{-1}$ (\pm SE, *n* = 6, *P* < 0.001). Thus, iodide at 0.1, 1.0 and 10 mM stimulated sulfate efflux (basal-to-peak) by 56%, 166.5% and 302.9% respectively. Iodide at a concentration of 50 mM in the incubation buffer increased the fractional release from $0.0116 \pm 0.0009 \text{ min}^{-1}$ to 0.0472 ± 0.0048 (\pm SE, *n* = 4, *P* < 0.01) (results not shown), therefore, it appears that the effect of iodide is maximal at a concentration of 10 mM. Figure 1 also shows the effect of 10 mM iodide on sulfate efflux in the presence of 1 mM DIDS. It is clear that DIDS completely inhibited the portion of sulfate efflux stimulated by external iodide. Furthermore, DIDS also inhibited sulfate efflux from mammary explants suspended in a gluconate buffer, thus, the fractional efflux was reduced (*P* < 0.001) from $0.0114 \pm 0.0003 \text{ min}^{-1}$ (\pm SE, *n* = 6) to $0.0025 \pm 0.0005 \text{ min}^{-1}$ (\pm SE, *n* = 4).

Shown for comparison in Fig. 1b is the effect of external sulfate on sulfate efflux from rat mammary tissue explants. It is clear that sulfate *trans*-stimulated the efflux of sulfate in a dose dependent manner. Sulfate at a concentration of 0.1 and 10 mM respectively increased the fractional release (basal-to-peak) by $0.0139 \pm 0.0008 \text{ min}^{-1}$ (\pm SE, *n* = 5, *P* < 0.001) and $0.0283 \pm 0.0032 \text{ min}^{-1}$ (\pm SE, *n* = 5, *P* < 0.001).

The Effect of Iodide on Sulfate Efflux from Rat Mammary Acini

The effect of external iodide (10 mM) in the absence and presence of DIDS (1 mM) on sulfate efflux from mammary acini is shown in Fig. 2. As with the experiments using explants, sulfate efflux was initially measured into a buffer containing gluconate followed by

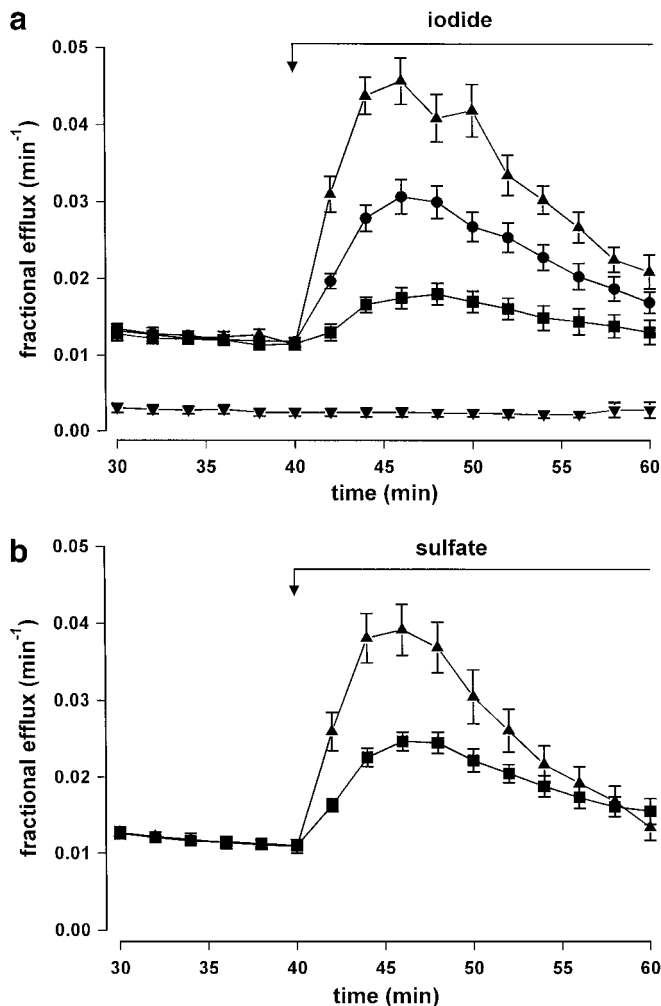


FIG. 1. (a) The effect of external iodide on sulfate efflux from rat mammary tissue explants. Sulfate efflux was first measured into a buffer containing (mM) 145 Na gluconate, 10 glucose, and 10 Tris-Mops, pH 7.4. At $t = 40$ (indicated by the bar) the tissue was incubated in a buffer containing (mM) 135–144.9 Na gluconate, 10 glucose and 10 Tris-Mops, pH, 7.4, plus 0.1 (■) ($n = 3$), 1.0 (●) ($n = 9$), or 10.0 (▲) ($n = 6$) NaI. The points denoted (▼) represent sulfate efflux in the absence and presence of 10 mM NaI in the presence of 1 mM DIDS ($n = 4$). Each point represents the mean \pm SE using tissue from separate animals for each experiment. Efflux was measured at 20°C. (b) The effect of external sulfate on sulfate efflux from rat mammary tissue explants. Sulfate efflux was initially measured into a buffer containing (mM) 145 Na gluconate, 10 glucose, and 10 Tris-Mops, pH 7.4. At $t = 40$ min (indicated by the bar) the tissue was incubated in a buffer containing (mM) 130–144.8 Na gluconate, 10 glucose, and 10 Tris-Mops, pH 7.4, plus 0.1 Na₂SO₄ (■) or 10 Na₂SO₄ (▲). Each point is the mean \pm SE of 5 experiments using tissue from separate animals. Efflux was measured at 20°C.

one supplemented with iodide. It is evident from Fig. 2 that external iodide increased the fractional efflux of sulfate (basal-to-peak) from $0.034 \pm 0.003 \text{ min}^{-1}$ to 0.103 ± 0.002 (\pm SE, $n = 3$, $P < 0.001$) an increase of 202%. DIDS completely inhibited the moiety of sulfate efflux induced by iodide. In addition, DIDS inhibited sulfate efflux ($P < 0.01$) from mammary acini incu-

bated in a buffer containing gluconate but no iodide: the fractional efflux at $t = 18$ min was reduced from $0.034 \pm 0.003 \text{ min}^{-1}$ to $0.015 \pm 0.0013 \text{ min}^{-1}$ (\pm SE, $n = 3$).

The Effect of Perchlorate (ClO_4^-) on Sulfate Efflux from Mammary Tissue Explants

Figure 3 illustrates the effect of perchlorate (1 mM) in the absence and presence of DIDS (1mM) from lactating rat mammary tissue explants. It is clear that perchlorate *trans*-stimulated sulfate efflux: the fractional efflux was increased (basal-to-peak) from $0.0101 \pm 0.0008 \text{ min}^{-1}$ to $0.0208 \pm 0.0020 \text{ min}^{-1}$ (\pm SE, $n = 4$, $P < 0.01$). The inclusion of DIDS in the incubation medium inhibited the moiety of sulfate efflux stimulated by perchlorate. Furthermore, in accordance with the results shown in Fig. 1a DIDS also inhibited sulfate efflux from tissue incubated in a gluconate buffer.

DISCUSSION

The results show that sulfate efflux from mammary tissue explants and acini isolated from rats during peak lactation was *trans*-stimulated by iodide in a dose-dependent fashion. Even though the transport of iodide was not measured directly the finding that external iodide stimulated sulfate efflux is evidence that iodide was indeed transported into mammary cells. The possibility that iodide-dependent sulfate efflux is via the Na^+/I^- cotransporter can be ruled out on the

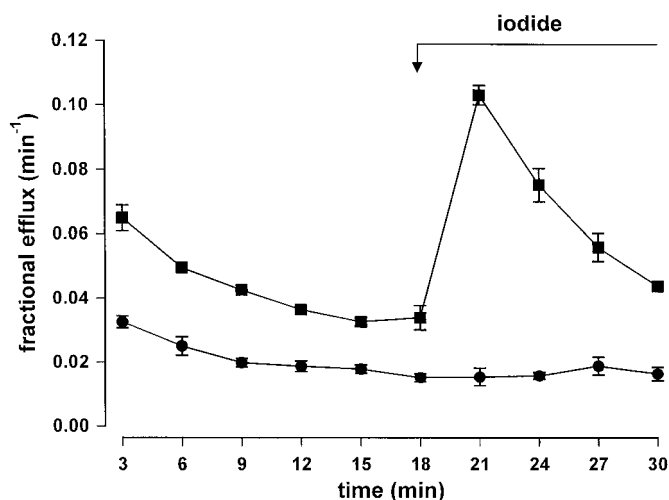


FIG. 2. The effect of iodide in the absence (■) and presence (●) of DIDS (1 mM) on sulfate efflux from rat mammary acini. Sulfate efflux was first measured into a buffer containing (mM) 145 Na gluconate, 10 glucose, and 10 Tris-Mops, pH 7.4. At $t = 18$ (denoted by the bar) the acini were suspended in a buffer containing (mM) 135 Na gluconate, 10 NaI, 10 glucose, and 10 Tris-Mops, pH 7.4. Each point is the mean \pm SE of 3 experiments using acini prepared from separate animals for each experiment. Efflux was measured at 20°C.

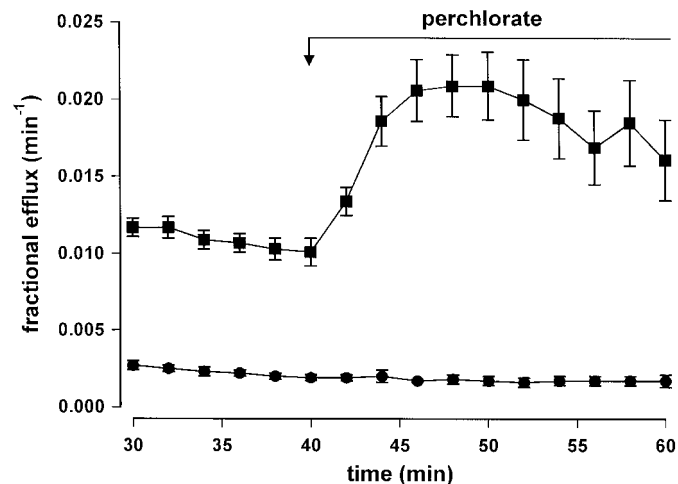


FIG. 3. The effect of perchlorate (1 mM) on sulfate efflux from rat mammary tissue explants in the absence (■) and presence (●) of DIDS (1 mM). Sulfate efflux was first measured into a buffer containing (mM) 145 Na gluconate, 10 glucose, and 10 Tris-Mops, pH 7.4. At $t = 40$ min (indicated by bar) the tissue was transferred into a buffer containing (mM) 1 NaClO₄, 144 Na gluconate, 10 glucose and 10 Tris-Mops, pH 7.4. Each point is the mean \pm SE for 4 and 3 experiments respectively in the absence and presence of DIDS. Efflux was measured at 20°C.

basis that the Na⁺-dependent system does not accept sulfate as a substrate (16).

The present findings are consistent with the notion that mammary tissue expresses an anion-exchange mechanism which accepts iodide as a substrate. This suggestion is strengthened by the observation that iodide-dependent sulfate efflux was inhibited by DIDS: this stilbene derivative is a known inhibitor of anion exchange processes (17). The anions which can utilize the exchanger are not limited to sulfate and iodide: it has previously been reported that sulfate efflux from rat mammary tissue explants can be *trans*-stimulated by chloride in a manner sensitive to DIDS (14). In addition, the present findings also suggest that perchlorate, commonly used as an inhibitor of the Na⁺/I⁻ cotransporter, is a substrate of the anion-exchange system. Iodide-sensitive sulfate efflux could be detected using mammary acini suggesting that the transporter resides in mammary secretory cells. Moreover, the presence of iodide-sensitive sulfate efflux in mammary explants suggests that the transport system may be located on the basolateral pole of the mammary epithelium.

Rillema and Rowady (8) found that mouse mammary tissue is able to concentrate iodide with respect to the incubation medium in the absence of external Na⁺. Thus, in the presence and absence of Na⁺ (choline replacement) the intracellular/extracellular distribution ratio of I⁻ was respectively 11.5 and 5.08. The possibility exists that iodide could be concentrated in exchange for other anions. However, it must be borne

in mind that other systems yet to be identified may also be responsible for concentrating iodide within mammary cells in the absence of extracellular Na⁺.

The exact contribution of iodide uptake via the sulfate/iodide exchange system to total iodide uptake by the gland remains to be determined. Nevertheless, the results of the present study suggest that iodide uptake into mammary cells does not solely rely on Na⁺-I⁻ cotransport. In view of this, the existence of more than one pathway for iodide uptake into mammary cells must be taken into account if strategies are to be devised to treat breast cancer with radiolabeled iodide.

Finally, it is notable that sulfate efflux from mammary explants and acini was also inhibited by DIDS when the incubation medium contained gluconate but no iodide. There are several explanations for this. First, gluconate, although believed to be impermeable, may be able to exchange for sulfate in mammary tissue. There is evidence to suggest that gluconate may exchange with Cl⁻ via the murine AE2 expressed in *Xenopus* oocytes (18). Second, as the buffers were only nominally free of bicarbonate, there is the possibility that there was sufficient bicarbonate present to *trans*-stimulate sulfate efflux. Third, the DIDS-sensitive efflux of sulfate into a gluconate medium could represent SO₄²⁻/OH⁻ exchange (19).

ACKNOWLEDGMENTS

This study was funded by the Scottish Executive Rural Affairs Department. The author is grateful to Mrs. Jean Thomson for expert technical assistance.

REFERENCES

- Brown-Grant, K. (1961) Extrathyroidal iodide concentrating mechanisms. *Physiol. Rev.* **41**, 189–213.
- De La Vieja, A., Dohan, O., Levy, O., and Carrasco, N. (2000) Molecular analysis of the sodium/iodide symporter: Impact on thyroid and extrathyroid pathophysiology. *Physiol. Rev.* **80**, 1083–1105.
- Shennan, D. B., and Peaker, M. (2000) Transport of milk constituents by the mammary gland. *Physiol. Rev.* **80**, 925–951.
- Tronko, M. D., Bogdanova, T. I., Komissarenko, I. V., Epstein, O. V., Oliynk, V., Kovalenko, A., Likhtarev, I. A., Kairo, I. Peters, S. B., and LiVolsi, V. A. (1999) Thyroid carcinoma in children and adolescents in Ukraine after the Chernobyl nuclear accident: Statistical data and clinicomorphologic characteristics. *Cancer* **86**, 149–156.
- Tuttle, R. M., and Becker, D. V. (2000) The Chernobyl accident and its consequences: Update at the millenium. *Semin. Nucl. Med.* **30**, 133–140.
- Tazebay, U. H., Wapnir, I. L., Levy, O., Dohan, O., Zuckier, L. S., Zhou, Q. H., Deng, H. F., Ameta, P. S., Fineburg, S., Pestell, R. G., and Carrasco, N. (2000) The mammary gland iodide transporter is expressed during lactation and in breast cancer. *Nature Med.* **6**, 871–878.
- Lengeman, F. W. (1973) Reduction of iodine transfer to milk of cows after perchlorate ingestion. *J. Dairy Sci.* **56**, 753–756.

8. Rillema, J. A., and Rowady, D. L. (1997) Characteristics of the prolactin stimulation of iodide uptake into mouse mammary gland explants. *Proc. Soc. Exp. Biol. Med.* **215**, 366–369.
9. Rillema, J. A., and Yu, T. X. (1996) Prolactin stimulation of iodide uptake into mouse mammary gland explants. *Am. J. Physiol.* **271**, E879–E882.
10. Rillema, J. A., Yu, T. X., and Jhiang, S. M. (2000) Effect of prolactin on sodium iodide symporter expression in mouse mammary gland. *Am J. Physiol.* **279**, E769–E772.
11. Dai, G., Levy, O., and Carrasco, N. (1996) Cloning and characterization of the thyroid iodide transporter. *Nature* **379**, 468–460.
12. Spitzweg, C., Joba, W., Eisenmenger, W., and Heufelder, A. E. (1998) Analysis of human sodium iodide symporter gene expression in extrathyroidal tissues and cloning of its complimentary deoxyribonucleic acids from salivary gland, mammary gland and gastric mucosa. *J. Clin. Endocrinol. Metab.* **83**, 1746–1751
13. Cho, J. Y., Leveille, R., Kao, R., Rousset, B., Parlow, A. F., Burak, W. E., Mazzaferri, E. L., and Jhaing, S. M. (2000) Hormonal Regulation of radioiodide uptake activity and Na^+/I^- symporter expression in mammary glands. *J. Clin. Endocrinol. Metab.* **85**, 2936–2943.
14. Shennan, D. B. (1989) A study of sulphate transport by lactating rat mammary tissue: Evidence for anion exchange. *Comp. Biochem. Physiol.* **92A**, 145–150.
15. Shennan, D. B., McNeillie, S. A., and Curran, D. E. (1994) The effect of a hyposmotic shock on amino acid efflux from lactating rat mammary tissue: Stimulation of taurine and glycine efflux via a pathway distinct from anion exchange and volume-activated anion channels. *Exp. Physiol.* **79**, 797–808.
16. Eskandari, S., Loo, D. D., Dai, G., Levy, O., Wright, E. M., and Carrasco, N. (1997) Thyroid Na^+/I^- symporter. Mechanism, stoichiometry and specificity. *J. Biol. Chem.* **272**, 27230–27238.
17. Cabantchik, Z. I., and Greger, R. (1992) Chemical probes for anion transporters of mammalian membranes. *Am. J. Physiol.* **262**, C803–C827.
18. Humphreys, B. D., Jiang, L., Chernova, M. N., and Alper, S. L. (1994) Functional characterization and regulation by pH of murine AE2 anion exchanger expressed in *Xenopus* oocytes. *Am. J. Physiol.* **267**, C1295–C1307.
19. Schron, C. M., Knickelbein, R. G., Aronson, P. S., Della Puca, J., and Dobbins, J. W. (1985) pH gradient-stimulated sulfate transport by rabbit ileal brush border membrane vesicles: Evidence for $\text{SO}_4\text{-OH}$ exchange. *Am. J. Physiol.* **249**, G607–G613.