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Sodium/iodide-symporter: distribution in different mammals and role in entero-thyroid circulation of iodide

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ABSTRACT

The sodium (Na⁺)/iodide (I⁻)-symporter (NIS) is abundantly expressed and accumulates iodide in thyroid follicular cells. The NIS is also found in extrathyroidal tissues, particularly gastric mucosa. Controversies exist on the localization of extrathyroidal NIS. We have studied the presence of both NIS peptide and NIS messenger RNA (mRNA) in the digestive tract and thyroid from different mammals. The role of gastric NIS is enigmatic and we aimed to unravel its possible involvement in iodide transport. Methods: Distribution and expression of NIS were studied using immunocytochemistry and *in situ* hybridization. Iodide transport in the gastrointestinal tract was measured after oral or intravenous (i.v.) administration of ¹²⁵I to rats with or without ligation of the pylorus. Results: All thyroid follicular cells in rat and mouse expressed NIS, whereas a patchy staining was noted in man, pig and guinea-pig. Gastric mucosa surface epithelium in all species and ductal cells of parotid gland in guinea-pig, rat and mouse expressed NIS. In parietal cells and in endocrine cells of intestines and pancreas NIS immunoreactivity but no NIS mRNA was found. Studies of ¹²⁵I uptake showed marked iodide transport from the circulation into the gastric lumen. Conclusions: The localization of NIS varies slightly among mammals. To establish expression of NIS in a particular cell type the need to correlate the presence of both NIS protein by immunocytochemistry and NIS mRNA by *in situ* hybridization is emphasized. An entero-thyroidal circulation of iodide mediated principally by gastric NIS, but possibly also by NIS in salivary glands is suggested.

Keywords entero-thyroid circulation, gastric mucosa, iodide transport, NIS, salivary glands, thyroid gland.

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Iodide is an essential component of thyroid hormones. It is absorbed from the gastrointestinal tract and highly concentrated in the follicular cells of the thyroid. The mechanisms governing iodide transport over cell membranes in the thyroid and in the gastrointestinal tract are not known in detail.

The sodium (Na⁺)/iodide (I⁻)-symporter (NIS) is a transmembrane protein mediating iodide transport into the follicular cells of the thyroid gland. As the complementary DNA (cDNA) sequences of rat NIS in a thyroid derived cell line (FRTL) (Dai *et al.* 1996) and human NIS in papillary thyroid carcinoma (Smanik *et al.* 1996) were revealed, studies on iodide transport and NIS protein structure as well as function have been vitalized (Nilsson 1999, de la Vieja *et al.* 2000).

The NIS has been detected by immunocytochemistry in rat thyroid (Paire *et al.* 1997) and gastric mucosa

(Kotani *et al.* 1998). In addition, reverse transcriptase-polymerase chain reaction (RT-PCR) technique has demonstrated the presence of NIS mRNA in rat brain, stomach, duodenum, skin, mammary gland, placenta, uterus and ovary but not in salivary glands (Kotani *et al.* 1998). In man, NIS-immunoreactive material has been found in thyroid follicular cells, gastric and large intestinal mucosa, ductal cells of salivary glands, lachrymal gland and some epithelial cells of mammary gland (Jhiang *et al.* 1998, Spitzweg *et al.* 1999, Vayre *et al.* 1999). Spitzweg *et al.* (1999) also found NIS-immunoreactive material in pancreatic endocrine and exocrine cells while in the study by Vayre *et al.* (1999) no NIS immunoreactivity could be detected in pancreas. Presence of NIS messenger RNA (mRNA), as demonstrated by RT-PCR technique, has been reported in human pituitary, thyroid, salivary glands, gastric

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mucosa, pancreas, adrenal gland, heart, lung, thymus, ovary, mammary gland, testis and prostate (Spitzweg *et al.* 1998).

In man thyroid and gastric NIS cDNA has been found to be identical (Vayre *et al.* 1999), while rat gastric NIS cDNA has been found to differ from rat thyroid NIS cDNA by three nucleotide substitutions leading to two amino acid substitutions in the NIS protein (Kotani *et al.* 1998). Gastric NIS has been suggested to be of an immature form, easily degraded and functioning to trap iodide from the gastric lumen although not very efficiently (Kotani *et al.* 1998).

The aims of this study were to examine the localization and the expression of NIS and NIS mRNA in the thyroid and digestive tract from different mammals and the role of gastric NIS for iodide transport in the gastrointestinal tract.

MATERIALS AND METHODS

Animals

A total of five female NMRI mice (20–30 g, Alab, Stockholm, Sweden), 24 female Sprague–Dawley rats (170–200 g, Alab, Stockholm, Sweden) and three female Dunkin–Hartley guinea-pigs (250 g, Sahlins, Malmö, Sweden) were used. The animals had free access to standard food pellets and tap water. Animals were killed by bleeding from a cardiac incision during deep diethyl ether or chloral hydrate anaesthesia. Specimens were also collected immediately postmortem from three pigs used for educational purposes at the University of Lund. Animal care was in accordance with the European Council Convention of 1986 and the study was approved by the research animal ethics committee at the University of Lund.

Human material

Normal thyroid and gastric tissues were obtained from patients undergoing thyroid surgery ($n = 4$) or gastrectomy ($n = 4$). The procedures were approved by the ethical committee at the University of Lund.

Specimens and tissue processing for microscopic examination

Thyroid and stomach (oxyntic and pyloric region; from rat and mouse also pars proventricularis) from all species as well as parotid and submandibular glands from guinea-pig, rat and mouse were examined. In addition specimens from oesophagus, duodenum, jejunum, colon descendens, rectum and pancreas from rat were included. Specimens were fixed in Stefanini's fixative (2% formaldehyde and 0.15% picric acid in phosphate buffer, pH 7.2) for 12–24 h and then repeatedly rinsed in

Tyrode buffer containing 10% sucrose, all at 4 °C. Specimens were frozen on dry ice, sectioned in a cryostat at –20 °C to a thickness of 10 µm, and thaw-mounted onto Superfrost plus slides (Tamro lab AB, Mölndal, Sweden). The sections were then processed either by immunocytochemistry or by *in situ* hybridization.

Immunocytochemistry

A peptide containing the eight C-terminal amino acids of rat-NIS (GHDVETNL) was synthesized in a SYRO multisynthesizer (Multisyn Tech, GmbH). Purity as analysed by high-performance liquid chromatography (HPLC) was >60%. The rationale for choosing the C-terminal as antigen was that it is located intracellularly, thus well preserved during tissue processing and that it has no significant homologies with other known proteins, including the major thyroid antigens and transporters (Benvenga *et al.* 1999, GeneBank, NCBI, sequence similarity search, www.ncbi.nlm.nih.gov/BLAST). The N-terminus of the peptide was conjugated to bovine serum albumin (BSA) and an antiserum was raised in rabbit using the peptide-BSA conjugate together with Freund's complete adjuvans. Sections were incubated with the primary antiserum (code number 9806, Euro-Diagnostica AB, Malmö, Sweden) diluted 1 : 1600 in BSA-containing phosphate buffered saline (PBS) over night in a moist chamber at 4 °C. In addition an affinity purified, polyclonal rabbit antiserum (TIT11-A, Alpha Diagnostic Intl. Inc., San Antonio, TX, USA) raised against the 16 C-terminal amino acids of rat NIS was used in dilution 1 : 160. The site of the antigen–antibody reaction was revealed by fluorescein isothiocyanate (FITC)-labelled pig antirabbit immunoglobulin G (IgG) (Dako, Copenhagen, Denmark) diluted 1 : 80. Incubation time with FITC-labelled antibody was 45–60 min in room temperature. For control purposes, the primary antisera were incubated with 100 µg mL⁻¹ of the respective synthetic peptide overnight at 4 °C before use.

In situ hybridization

For the detection of NIS mRNA a 33-mer oligonucleotide probe complementary to rat thyroid NIS mRNA 570–602 (TCATCCTGAACCAAGTGACCGGGTTGGACATCT) (Dai *et al.* 1996) was constructed (Biomolecular Resource Facility, University of Lund, Sweden). The probe was 3'-endtailed with ³⁵S-deoxyadenosine triphosphate (dATP) by use of terminal transferase (both supplied by NEN, DuPont, Stockholm, Sweden), yielding a specific activity of approximately 2 × 10⁹ CPM µg⁻¹. Cryostat sections were prepared as described previously. The hybridization protocol has previously been described in detail (Mulder

et al. 1994). Briefly, after clearing in chloroform, the sections were acetylated and hybridized with the probe (1 pmol mL^{-1}) overnight at 37°C . The slides were washed in saline sodium citrate ($4 \times 15 \text{ min}$, 55°C), dehydrated and dipped in emulsion (Ilford K5, Mettssons Foto AB, Sweden). Exposure time was 5 weeks, after which the slides were developed in Kodak D-19 (Gerh. Ludwigsen, Partille, Sweden). For control purposes, hybridization was also performed after incubation in ribonuclease (RNase) A ($45 \mu\text{g mL}^{-1}$, Sigma, St Louis, MO, USA; 30 min at 37°C) or in the presence of a 100-fold excess of unlabelled probe in the hybridization buffer.

Radioidide uptake in rat

The ^{125}I ($25 \mu\text{Ci}$ in $0.5\text{--}1 \text{ mL}$ of saline) was administered to rats either intravenously (i.v.) or orally. All rats were fasted overnight before experimentation. Rats appointed to receive ^{125}I orally ($n = 7$) were anaesthetized with diethyl ether and the abdomen was opened. In four of these rats pylorus was ligated and in the other three left unligated. The abdomen was closed and the rats were allowed to wake up before placing the gastric catheter for iodide administration. Rats appointed to receive ^{125}I i.v. ($n = 6$) were anaesthetized with chloral hydrate (300 mg kg^{-1} intraperitoneally), the abdomen was opened, pylorus was ligated in three of the rats and left unligated in the other three. The abdomen was then closed and ^{125}I was injected into the femoral vein. Sixty minutes after iodide administration orally or i.v., 0.3 mL blood was collected by cardiac puncture under chloral hydrate anaesthesia, after which the rats were killed by bleeding. The blood samples were put directly in test tubes with 0.7 mL of saline. Thyroid–larynx complex, stomach, jejunum (approximately 2 cm), colon descendens (approximately 2 cm), and kidney were removed and weighed. All stomachs were ligated at both ends before removal and the stomachs as well as the intestines were cut open and washed in 8 mL of physiological saline for 1 min before measurements. The washing fluids obtained by this procedure from stomach and colon of i.v. treated rats (with and without pyloric ligation) were used for analysis of luminal ^{125}I content. Blood samples, washing fluids, and excised organs were measured for radioactivity in an automatic NaI(Tl) scintillation well counter (1282 Compugamma; LKB, Finland) and radioactivity was expressed as percentage of total administered ^{125}I per organ or sample.

Statistics

Values are single observations or ranges. For statistical analysis, values of radioactivity for washing fluids were expressed as ratio of washing fluid radioactivity to

corresponding organ sample radioactivity. This was to compensate for sample size differences and a possible leakage of ^{125}I from the tissue. Comparisons were made with Wilcoxon's signed rank test.

RESULTS

Localization of NIS

Two different antisera were tested. The NIS antiserum 9806 resulted in strong and intense immunolabelling of the thyroid glands in all species studied, whereas the immunolabelling obtained by TIT-11A was weak and restricted to rat and mouse thyroid glands. The staining pattern obtained using the TIT-11 A antibodies was identical to that of antiserum 9806 in that the thyroid follicles possessed a basolateral immunolabelling. The immunolabelling was abolished by pre-incubating the antisera (both 9806 and TIT-11 A) with excess peptide. The TIT-11A failed to demonstrate NIS immunoreactivity in human, pig and guinea-pig thyroid glands. Therefore, the immunocytochemical results are mainly based on the NIS antiserum 9806.

The oligonucleotide probe generated for *in situ* hybridization showed consistent autoradiographic labelling in guinea-pig, rat and mouse thyroid gland, gastric mucosa and salivary glands demonstrating presence of NIS mRNA. In these locations no autoradiographic labelling was obtained in the control experiments. In man and pig no autoradiographic labelling was detected in any of the specimens including thyroid, indicating that the probe does not recognize the NIS mRNA sequences of these species.

Thyroid gland. In general NIS immunoreactivity predominated basolaterally in the follicular cells (Fig. 1a). This staining pattern was less obvious in man (Fig. 1b) and pig. In these two species some follicular cells displayed a basolateral localization of NIS, but a large number of the NIS-immunoreactive cells were diffusely stained throughout the cytoplasm. In rat (Fig. 1a) and mouse virtually all follicular cells were stained, whereas in man (Fig. 1b), pig and guinea-pig the NIS-immunoreactive material was unevenly distributed within follicles and the number of stained cells varied between follicles. *In situ* hybridization on rat (Fig. 2a) and mouse thyroid gland showed intense autoradiographic labelling representing the presence of NIS mRNA, which corresponded to the immunocytochemical findings in that the bulk of follicular cells expressed NIS. In guinea-pig the distribution of NIS mRNA was patchy (Fig. 2b) as was the immunocytochemical staining.

Stomach. In the oxyntic and pyloric mucosa of man, rat and mouse, intense NIS immunoreactivity was

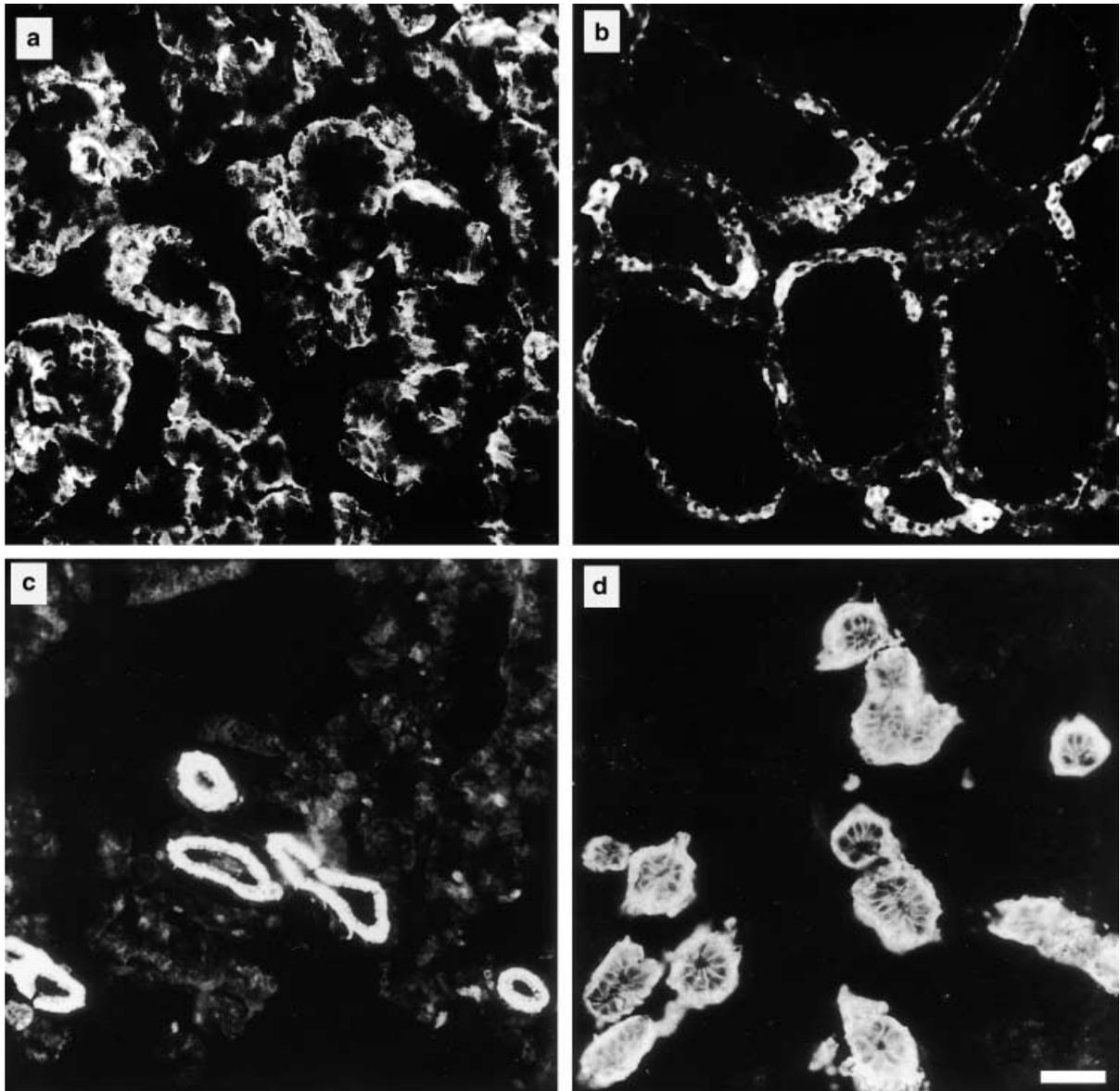


Figure 1 Cryostat sections of rat thyroid (a), human thyroid (b), mouse parotid gland (c) and guinea-pig parotid gland (d) immunostained for NIS. The NIS immunoreactivity is preferentially localized basolaterally in thyroid follicular cells. The NIS is present in virtually all follicular cells in rat, but has a more patchy distribution in man. In parotid gland NIS immunoreactivity is contained within the ductal cells and mainly found basolaterally. The scale bar in (d) represents 80 μm (a, b and d) or 125 μm (c).

found basolaterally in the epithelial surface cells including the epithelial cells lining the gastric pits (Fig. 3). NIS immunoreactivity of moderate intensity was also noted in the mucosal surface epithelium (including gastric pits) in pig and guinea-pig. In addition strong NIS immunoreactivity was noted in a large number of the parietal cells in man (Fig. 3c) and to a lesser extent in pig, guinea-pig and mouse (Fig. 3a), whereas such staining was absent in rat (Fig. 3b). In the pars proventricularis no NIS immunoreactivity was found in rat or mouse (the only two species studied possessing this gastric portion). Autoradiographic labelling of

NIS mRNA corresponded well to the pattern of immunoreactivity seen in guinea-pig, rat (Fig. 2c) and mouse in that a strong autoradiographic labelling of the epithelium was detected. The parietal cells in guinea-pig, rat and mouse expressed no NIS mRNA as studied by *in situ* hybridization.

Oesophagus and intestines. An extended investigation on the presence of NIS and NIS mRNA in oesophagus, duodenum, jejunum, colon and rectum was undertaken in rat. In the small intestine a few scattered NIS-immunoreactive endocrine cells were found, but these

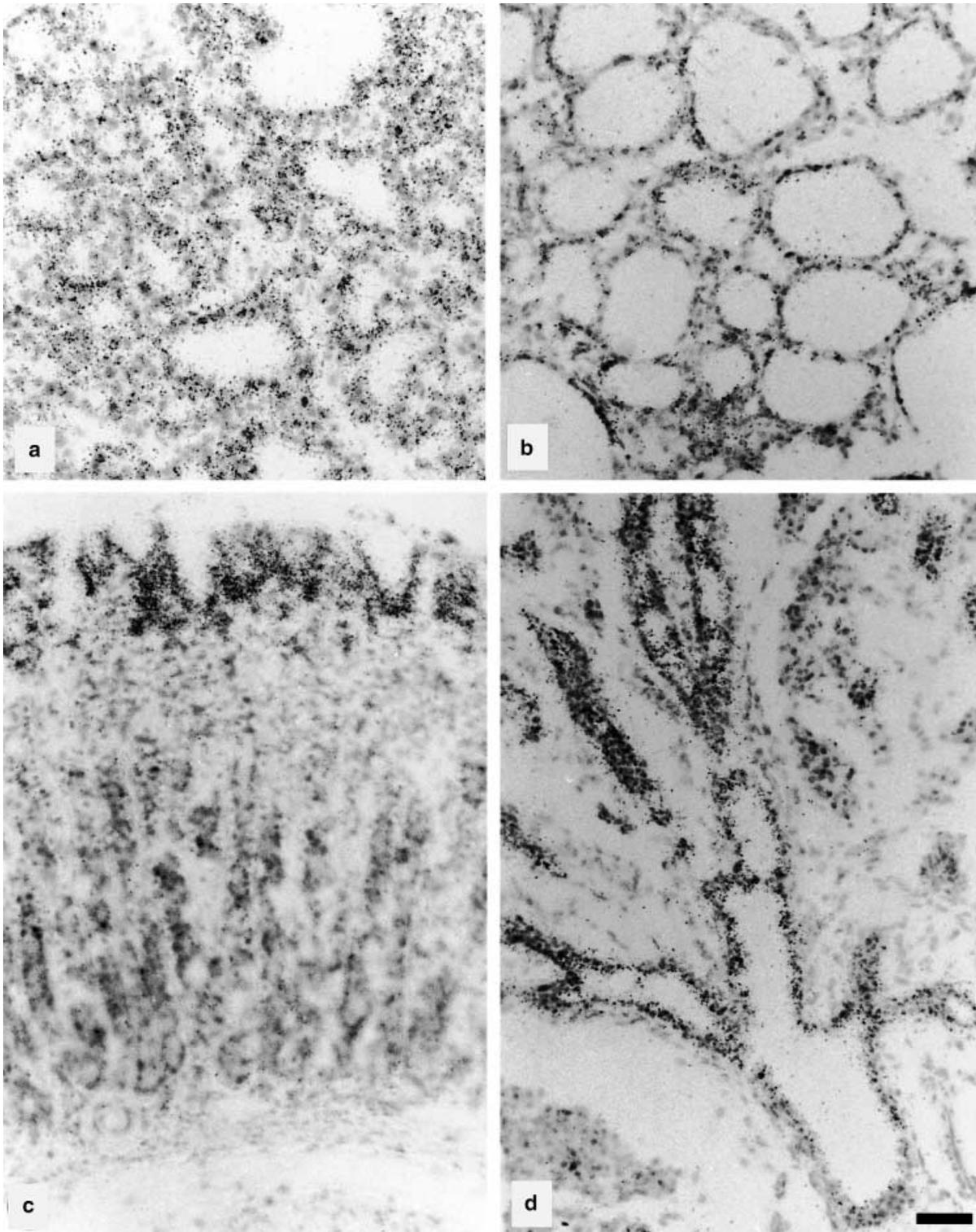


Figure 2 Cryostat sections of rat thyroid (a), guinea-pig thyroid (b), rat stomach oxyntic mucosa (c) and guinea-pig parotid gland (d) autoradiographically labelled for NIS mRNA. The labelling is homogeneously distributed in rat thyroid follicular cells (a) but patchy in guinea-pig thyroid (b). Intense labelling is seen in the gastric surface epithelium (c) and in ductal cells of the parotid gland (d). The scale bar in (d) represents 80 μm (a–c) or 60 μm (d).

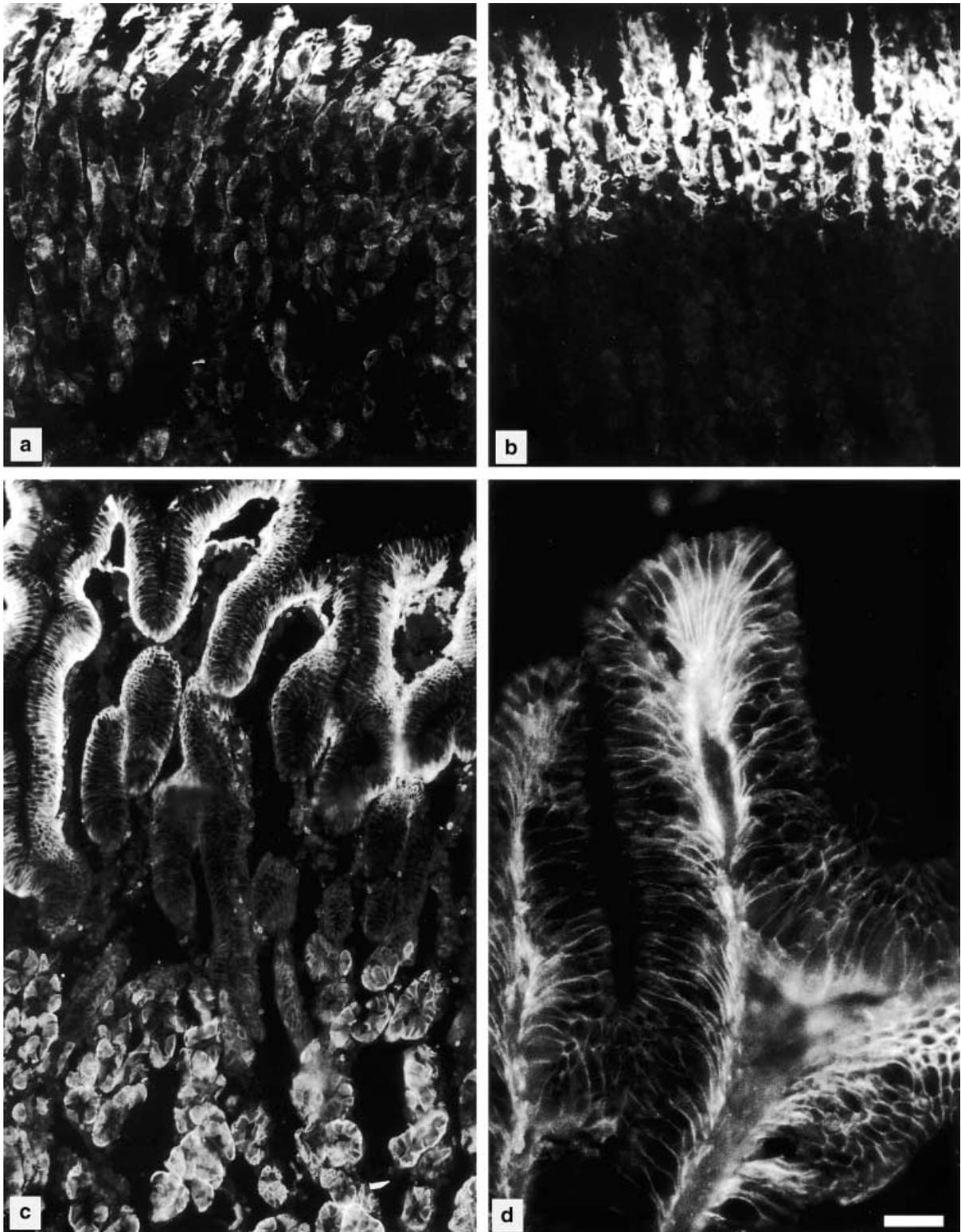


Figure 3 Cryostat sections of gastric oxyntic mucosa from mouse (a), rat (b) and man (c and d) immunostained for NIS. The NIS-immunoreactive material is found basolaterally in epithelial surface cells (a–d) and localized to the cell membrane in parietal cells (a and c). The scale bar in (d) represents 80 μm (a and b), 125 μm (c) or 50 μm (d).

cells showed no detectable labelling of NIS mRNA by *in situ* hybridization. No NIS-immunoreactive material or NIS mRNA could be detected in the oesophagus or large intestine.

Salivary glands. In rat, weak NIS immunoreactivity could be detected in the ductal cells of the parotid gland, whereas in guinea-pig and mouse parotid glands NIS immunoreactivity was intense basolaterally in the ductal cells (Fig. 1c, d). In mouse submandibular gland intense NIS immunoreactivity was found in both intra- and interlobular ducts, while in the sublingual gland only a weak immunostaining of NIS could be detected in ductal cells. No NIS-immunoreactive material could be detected either in guinea-pig or in rat submandibular gland. The NIS mRNA labelling by *in situ* hybridization was barely detectable in rat parotid ductal cells but strong in guinea-pig (Fig. 2d) and mouse parotid ductal cells, which are in accordance with the immunocytochemical findings.

Pancreas. In rat pancreas a moderate number of NIS-immunoreactive cells was found within the islets. Such cells displayed, however, no NIS mRNA labelling. No NIS immunoreactivity or NIS mRNA was detected in the exocrine parenchyma.

Radioiodide uptake in rat

With open (unligated) pylorus iodide uptake in the thyroid was high after both oral and i.v. administration of ^{125}I (Table 1). With closed (ligated) pylorus the thyroid uptake of ^{125}I after oral administration of iodide was almost negligible, whereas in rats receiving iodide i.v. pyloric ligation did not hamper uptake of ^{125}I (Table 1). The utterly low uptake of ^{125}I in the thyroid after oral administration with closed pylorus was accompanied by a low content of ^{125}I in blood compared with the other groups (Table 1) ($P = 0.006$). Presence of iodide within the gastric wall was of the same magnitude irrespective of iodide administration route or pyloric ligation (Table 1). Jejunum had no

obvious accumulation of iodide and kidneys consistently showed activity slightly above that of the blood samples in all four groups (Table 1).

After i.v. administration of ^{125}I , 9–16% of the administered dose was found in the stomach washing fluid irrespective of the pylorus being closed or open, whereas only 0.017–0.039% was found in the colon washing. For comparison of stomach and colon washing fluids all i.v. treated rats were considered as one group. The ratio of washing fluid radioactivity to corresponding organ sample radioactivity was clearly higher for stomach (1.9–3.2) than for colon (0.26–0.63) ($P = 0.03$).

DISCUSSION

Expression and distribution of NIS

The localization and expression of NIS in thyroid follicular cells is of great interest as NIS is a crucial protein for iodide trapping. The significance of NIS expression in other organs, such as the gastric mucosa, is less well understood. The present study verifies previous immunocytochemical observations on the presence of NIS in the gastric mucosa in man and rat, but also extends these studies to other species (pig, guinea-pig and mouse) as well as to other regions of the digestive tract. In addition we present a probe able to detect the expression of NIS mRNA by *in situ* hybridization in guinea-pig, rat and mouse, whereas it did not label NIS mRNA in pig or man.

The NIS immunoreactivity was predominantly localized basolaterally in thyroid follicular cells. This was, however, less obvious in pig and man, where many of the follicular cells displayed NIS immunoreactivity throughout the cytoplasm. Others have reported on a basolateral localization of NIS in normal human follicular cells (Jhiang *et al.* 1998, Castro *et al.* 1999, Vayre *et al.* 1999). This seems, however, to be a more prominent feature in Graves disease (Castro *et al.* 1999, Spitzweg *et al.* 1999), in which the expression of NIS is higher than normal. A strictly basolateral localization of

Table 1 Iodide uptake as percentage per organ of administered dose

Groups*	<i>n</i>	Thyroid	Stomach‡	Jejunum	Colon	Kidney	Blood (0.3 mL)
Oral	3	3.5–4.2	3.0–3.1	0.05–0.11	0.04–0.06	0.39–0.45	0.35–0.38
Oral + liga†	4	0.03–0.42	1.9–2.5	0.001–0.07	0.001–0.08	0.004–0.5	0.004–0.2
i.v.	3	1.2–2.1	3.9–4.7	0.05–0.07	0.04–0.11	0.47–0.62	0.27–0.47
i.v. + liga‡	3	1.1–2.7	4.3–5.6	0.06–0.24	0.05–0.08	0.49–0.66	0.31–0.54

*Groups are based on iodide administration route.

†Liga = pyloric ligation.

‡Glandular portion.

Values are ranges for each group.

NIS may be a feature only in active follicular cells. The NIS and NIS mRNA were expressed in virtually all follicular cells in mouse and rat, whereas in guinea-pig, pig and man NIS immunoreactivity was patchy, which, in man, is in accordance with the findings of others (Caillou *et al.* 1998, Castro *et al.* 1999). The patchy distribution of NIS may reflect differences in metabolic activity of the thyroid follicular cells in these species. In this context it is worth mentioning that organic iodide, as studied by analytical ion microscopy, has a similarly patchy distribution in normal human thyroid (Fragu *et al.* 1989). However, it is not clear whether the cells with a high content of organic iodide are the same as those rich in NIS.

Gastric NIS in rat has been cloned and the cDNA was found to be nearly identical with thyroid NIS. However, its post-translational modification (mainly glycosylation) differs from that of thyroid NIS (Kotani *et al.* 1998). In the present study on gastric mucosa, as well as in the study by Vayre *et al.* (1999), very intense NIS immunoreactivity was found in the basolateral cell membranes of the surface epithelium. This is in contrast to the findings of Kotani *et al.* (1998) who reported that NIS was preferentially located at the apical border of the gastric epithelium. In accordance with Spitzweg *et al.* (1999) we found NIS-immunoreactive material also within the parietal cells of the gastric mucosa in man. NIS-immunoreactive parietal cells were also detected in pig, guinea-pig and mouse (present study). The identity of the parietal cells was confirmed by costaining with an antiserum directed against H^+/K^+ -ATPase (data not shown). However, the presence of authentic NIS in these cells is strongly questioned as no NIS mRNA labelling could be detected. Thus, the possibility that the NIS-immunoreactive material in parietal cells represents cross reactivity with some other protein/transporter must be considered.

Presence of NIS and/or NIS mRNA has been reported in a large number of tissues, mainly by the use of RT-PCR technique (Kotani *et al.* 1998, Spitzweg *et al.* 1998). In accordance with previous findings in human salivary glands (Spitzweg *et al.* 1999) we found a high expression of NIS and NIS mRNA in salivary glands of guinea-pig and mouse, particularly within the ductal epithelium. The present finding of NIS immunoreactivity and NIS mRNA in ductal cells of rat parotid gland is noteworthy as it is in contrast to the negative findings on NIS expression in this location by Kotani *et al.* (1998). On the other hand we could not confirm the report of NIS expression in the cornification layer of rat pars proventricularis (Kotani *et al.* 1998). A novel finding reported in the present study was the detection of NIS-immunoreactive material in endocrine cells in the pancreas and small intestine of the rat which is in

line with the finding of NIS immunoreactivity in pancreatic endocrine cells in man (Spitzweg *et al.* 1999). However, our failure in detecting NIS mRNA expression in these cells, parallel to our findings in parietal cells, offers two possibilities, either a very low mRNA expression (below detection limit) or cross-reactivity of the antibodies with a related antigen. Conflicting reports on the localization of NIS can, at least partly, be the result of methodological sensitivity differences. The combined use of *in situ* hybridization and immunocytochemistry in order to establish the expression of both NIS mRNA and NIS protein is crucial in order to minimize false positive results.

Gastric NIS function The great abundance of NIS and NIS mRNA in gastric mucosa raises the question of its functional role in this location. In the present study, a considerable transport of iodide from the bloodstream to the gastric lumen could be established, whereas no indication of any substantial gastric luminal uptake of iodide was found. Our suggestion is that iodide transport to the gastric lumen from the circulation is the result of an active pumping mediated by NIS located within the gastric mucosa. As the absorption of iodide occurs in the small intestine (for a review see Brown-Grant 1961), gastric NIS is likely to play an important role in iodide conservation by an entero-thyroid recirculation. This is supported by the finding of a gastrointestinal recirculation of iodide in the dairy cow in which iodide re-entry from the circulation to the gastrointestinal tract was suggested to be mediated via secretion of iodide by the gastric mucosa of the abomasum (Miller *et al.* 1973 and 1975). In addition to gastric secretion a small amount of iodide is reported to enter the gastrointestinal tract via saliva particularly from parotid glands (Brown-Grant 1961). NIS-immunoreactive material has been detected in ductal cells of human salivary glands (Jhiang *et al.* 1998, Spitzweg *et al.* 1999) and this was found, in the present study, to apply also to guinea-pig, rat and mouse. In particular the parotid glands were found to express NIS, thus providing a morphological basis for the suggestion that salivary glands contribute to the recirculation of iodide. The secretion of iodide by exocrine glands and gastrointestinal mucosa may also have an antimicrobial function (Majerus & Courtois 1992) or act as an antioxidant (Venturi & Venturi 1999).

Whether the gastric mucosa, in addition to secreting iodide, is able to take up iodide as has been suggested (Kotani *et al.* 1998) is still an open question. Our findings of negligible uptake in blood and thyroid of ^{125}I given orally to rats with pylorus ligated argue against the gastric mucosa as a site for systemic iodide uptake.

Concluding remarks We suggest that NIS is not only of high importance for iodide trapping in the thyroid follicular cells, but also plays an important role in iodide conservation. By its presence in salivary glands and gastric mucosa, NIS mediates iodide transport from the circulation to the gastrointestinal tract via an active pumping of iodide to the lumen. The secreted iodide is then again transported to and taken up by the small intestine. An effective recirculation of iodide may thus protect against iodide deficiency.

Studies linking NIS directly to the secretion of iodide into the gastrointestinal tract as well as studies on the functional role of NIS expression in other extra thyroidal organs and the possibility that NIS may participate in the secretion of anions other than Γ^- such as ClO_3^- or NO_3^- would be of great interest. Also, in view of the findings that the incidence of (Holm *et al.* 1991) and the mortality in (Hall *et al.* 1992) gastric carcinoma seems to be elevated after ^{131}I -therapy, a better understanding of gastric iodide transport and the possible accumulation of iodide in this organ is desirable.

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