Biochimica et Biophysica Acta, 943 (1988) 43–52
Elsevier

BBA 74064

Taurine and cell volume maintenance in the shark rectal gland: cellular fluxes and kinetics

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(Received 26 January 1988)

Key words: Isoosmotic cell swelling; Cell swelling; Taurine uptake; Taurine efflux; Diuretic; β-Amino acid; *p*-Chloromercuribenzene sulfonate; (Shark rectal gland tissue)

Tissue slices of shark rectal gland are studied to examine the kinetics of the cellular fluxes of taurine, a major intracellular osmolyte in this organ. Maintenance of high steady-state cell taurine (50 mM) is achieved by a ouabain-sensitive active Na^+-dependent uptake process and a relatively slow efflux. Uptake kinetics are described by two saturable taurine transport components (high-affinity, K_m 60 μM; and low-affinity, K_m 9 mM). [^{14}C]Taurine uptake is enhanced by external Cl^-, inhibited by β-alanine and unaffected by inhibitors of the $Na^+/K^+/2Cl^-$ co-transport system. Two cellular efflux components of taurine are documented. Incubation of slices in *p*-chloromercuribenzene sulfonate (1 mM) reduces taurine uptake, increases efflux of taurine and induces cell swelling. Studies of efflux in isotonic media with various cation and anion substitutions demonstrate that high-K^+ markedly enhances taurine efflux irrespective of cell volume changes (i.e. membrane stretching is not involved). Moreover, iso-osmotic cell swelling induced in media containing propionate is not associated with enhanced efflux of taurine from the cells. It is suggested that external K^+ exerts a specific effect on the cytoplasmic membrane to increase its permeability to taurine.

Introduction

Taurine (β-aminoethanesulfonic acid) is an organic osmolyte which is present in relatively high concentrations in the cells of a variety of marine species. This amino acid is believed to contribute significantly to the maintenance and regulation of cell volume by counteracting the osmotic effects of high salt concentrations in extracellular fluid [1,2]. In many fish, taurine is derived predominantly from dietary sources, with little, if any, subsequent oxidation [3]. The characteristics of cellular taurine accumulation have previously been examined in many tissues of several species. In several marine and mammalian species, the uptake of taurine into the cell proceeds against a large concentration gradient involving a carrier-mediated system, driven largely by the inwardly-directed Na^+ gradient [2,4]. Similar information on taurine transport in the rectal gland of the spiny dogfish shark (*Squalus acanthias*) is lacking, although in this tissue taurine appears to be a major intracellular osmolyte [5]. The rectal gland of elasmobranchs consists of a

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Cl^--secreting epithelium which is composed of virtually a single-cell type.

In the current study, we utilized rectal gland slices to examine the kinetics of uptake and efflux of taurine. Evidence is provided for Na^+-dependent taurine uptake which can be enhanced by external Cl^- and inhibited by β-alanine. The data are consistent with the notion that in rectal gland cells the amino acid behaves as an effective intracellular osmolyte which is retained against a large concentration gradient. The efflux rate is relatively slow but can be markedly enhanced in high-K^+ media. However, this apparent augmentation in membrane permeability to taurine may not necessarily be linked to cell swelling or membrane stretching per se, but appears to reflect a specific effect of external K^+ on membrane properties.

A portion of this work was presented at the National Meeting of the American Federation for Clinical Research, Washington DC, April 1988, and appeared in Abstract form in Clin. Res. 36, 530 (1988).

Methods and Materials

Tissue and media. Rectal gland slices were obtained from the spiny dogfish shark (*Squalus acanthias*) as described elsewhere [6]. The standard incubation medium (elasmobranch Ringer's solution) contained (in mM): Na^+, 280; Cl^-, 285; K^+, 5.0; HCO_3^-, 8; Ca^{2+}, 2.5; Mg^{2+}, 1.2; phosphate, 1; sulfate, 0.5; urea, 350; acetate, 5; osmolality, 920 mosmol/kg H_2O; pH 7.6 when gassed with 1% CO_2 in air. Modifications of this medium were as follows: (a) Na^+-free media: equivalent exchange of Na^+ by Li^+, K^+ or choline; (b) Cl^--free media: equivalent exchange of Cl^- by gluconate or propionate; (c) K^+-gluconate media: substitution of Na^+ by K^+, and Cl^- by gluconate. In certain experiments, the Ringer's solution contained ^{14}C-labeled polyethylene glycol (PEG), mol.wt. 4000 (1 mg/ml; 0.1 μCi/ml) as a marker of the extracellular space (minimum incubation 90 min).

Taurine uptake. Incubation of tissue slices was carried out as previously described [6,7]. For the determination of taurine uptake, slices were incubated for 30–270 min at 15°C (1% CO_2 in air) in media containing [^{14}C]taurine (0.1 μCi/ml) and a variable concentration (S_o) of unlabeled taurine (0.02–12 mM). At the end of each experimental point, at least four samples of tissue were blotted, weighed (wet wt., WW) then extracted for 24 h with 0.5 ml 0.04 M nitric acid. Radioactivity of tissue extract and incubation media was counted by liquid scintillation for the calculation of taurine uptake, expressed in μmol/g wet wt. In some experiments, the tissue was dried for 24 h at 95°C to obtain dry weight (DW). Intracellular water content was determined from the difference between WW and DW of tissue, using ^{14}C-PEG to correct for fluid in the extracellular tissue space. The apparent intracellular concentration (S_i) of the ^{14}C-labeled taurine moiety trapped inside the cell was calculated from the difference between total tissue uptake of [^{14}C]taurine and the amount of taurine present in the extracellular tissue space, expressed in mmol per kg cell water (i.e. mM). The accumulation ratio S_i/S_o at the end of incubation was also calculated.

Taurine efflux. Tissue slices were first preloaded with the label ([^{14}C]taurine, 0.2 μCi/ml) in media containing 0.2 mM taurine by aerobic incubation in standard saline for 180 min. The washout of the label over 120 min was then carried out using the technique described elsewhere [6,8]. Groups of loaded slices were blotted, weighed and used (a) for the determination of the initial activity of the label in the tissue (in cpm/g WW); (b) for the washout in a series of 10 tubes, each containing 10 ml unlabeled standard (control) or modified saline (see Results). After the washout, the label was also determined in the blotted, weighed tissue in order to complete the balance sheet of activities recorded. The data were then plotted as log (% activity remaining in the tissue) as a function of time.

Materials. All reagents were standard analytical grade. ^{14}C-PEG and [^{14}C]taurine were purchased from New England Nuclear (Boston, MA) and Amersham (Arlington Heights, IL), respectively.

Statistics. Results are expressed as mean ± S.E. for the group. Statistical significance ($P < 0.05$) was determined by Student's *t*-test. Linear regression analysis of Eadie-Hofstee plots was used to determine kinetic parameters. Rate constants of efflux were derived graphically.

Results

1. Basal uptake

The time course of taurine uptake into rectal gland slices was nearly linear over 30–270 min of incubation in control media containing 0.2 mM taurine (Fig. 1). Considerable variations in the rate of uptake by individual fish were noted. The S_i/S_o ratio was above unity at 120 min of incubation in some glands, and at 180 min of incubation in all glands examined. Basal uptake of taurine at 180 min was 0.249 ± 0.019 μmol/g WW ($n = 10$) with a corresponding S_i/S_o of 2.02 ± 0.17. In all subsequent experiments, taurine uptake was determined at 180 min of incubation. Kinetic parameters of taurine uptake are shown in Table I. These values were obtained by examining uptake as a function of media taurine concentration in standard Ringer's solution followed by linear regression analysis of Eadie-Hofstee plots (Fig. 2).

TABLE I

KINETIC PARAMETERS OF TAURINE UPTAKE IN RECTAL GLAND SLICES

Taurine uptake was determined in rectal gland slices incubated in standard elasmobranch Ringer's with [^{14}C]taurine (0.1 μCi/ml) for 180 min. The taurine concentration varied between 0.02 and 12 mM. K_m and V_{max} values (±S.E.) were obtained by linear regression analysis of Eadie-Hofstee plots (Fig. 2), with each point the mean of four analyses.

	K_m	V_{max} (μmol/h per g WW)
High-affinity system	60±5 μM	0.167±0.02
Low-affinity system	9±1 mM	1.83 ±0.20

Two saturable kinetic systems were revealed: a high-affinity (K_m 60 μM), low-capacity system and a low-affinity (K_m 9 mM), high-capacity system (Table I).

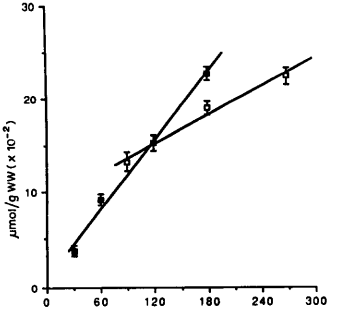

Fig. 1. The time course of taurine uptake in dogfish rectal gland slices is given for two representative glands. In one experiment (open squares, $n = 4$ slices), taurine uptake was determined between 90 and 270 min. In the other experiment (closed squares, $n = 3$ slices), uptake was measured between 30 and 180 min. The incubation medium was standard Ringer's solution containing [^{14}C]taurine (0.2 mM, 0.1 μCi/ml). Values are mean±S.E. (bars) for taurine uptake in μmol/g tissue wet weight (WW).

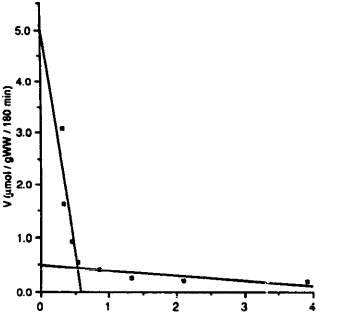

Fig. 2. The kinetics of taurine uptake (V) as a function of media taurine concentration (S) are examined using an Eadie-Hofstee plot. Each data point is the mean of four analyses. Taurine uptake was measured by incubating tissue slices for 180 min in standard media containing variable concentrations of taurine ranging between 0.02 and 12 mM. Linear regression analysis for best fit ($r > 0.77$) yielded two straight lines which describe two kinetic systems. Summary of the derived kinetic parameters are given in Table I.

TABLE II

Na^+-DEPENDENCY OF TAURINE UPTAKE IN RECTAL GLAND SLICES

Tissue was incubated for 180 min in different media containing 0.2 mM [^{14}C]taurine (0.1 μCi/ml). Taurine uptake (μmol/g WW ± S.E., n = 4–8) was examined in controls (C) (elasmobranch Ringer) or in modified Ringer (E) in which Li^+, choline or K^+ were substituted for Na^+; ouabain (0.5 mM) was added to standard, Na^+-containing Ringer. Mean values of the taurine accumulation ratio S_i/S_o are given.

	Li^+		Choline		K^+		Ouabain	
	C	E	C	E	C	E	C	E
Taurine uptake (μmol/g WW)	0.319 ±0.033	0.050 * ±0.006	0.403 ±0.002	0.065 * ±0.003	0.252 ±0.009	0.033 * ±0.004	0.404 ±0.002	0.299 ** ±0.016
S_i/S_o	2.62	0.15	3.33	0.19	2.01	0.01	3.33	2.42

*P < 0.001 and **P < 0.005 when the value in E is compared with the corresponding value in C.

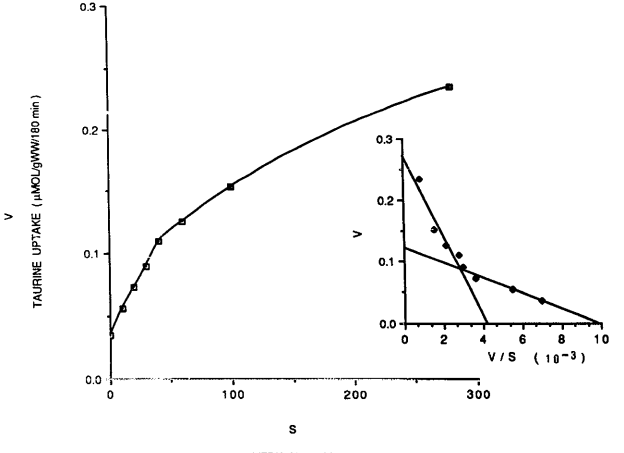

Fig. 3. The profile of taurine uptake (V) as a function of media Na^+ concentration (S) demonstrates saturation kinetics. Each data point is the mean of four analyses. Taurine uptake was measured by incubating tissue slices for 180 min in media containing 0.2 mM taurine and varying concentrations of Na^+ from 1 to 280 mM (Li^+ was used for iso-osmotic Na^+ substitution). The inset shows an Eadie-Hofstee plot with two straight lines drawn by linear regression for best fit. The slopes yield two affinities for Na^+ with K_m values of 12 ± 2 and 64 ± 4 mM. (Alternatively, the points can be fitted for a single straight line (r = 0.80); here the apparent affinity for Na^+ is 31 ± 3 mM).

TABLE III

EFFECT OF Cl^--SUBSTITUTION ON TAURINE UPTAKE IN RECTAL GLAND SLICES

For experimental conditions, see legend to Table II. E is modified Ringer in which gluconate ($n = 12$) or propionate ($n = 4$) is substituted for Cl^-.

	Na^+-gluconate		Na^+-propionate	
	C	E	C	E
Taurine uptake (μmol/g WW)	0.228 ±0.019	0.158 * ±0.004	0.199 ±0.015	0.138 ** ±0.005
S_i/S_o	1.79	0.96	1.46	0.93

* $P < 0.025$ and ** $P < 0.005$ when the value in E is compared with the corresponding value in C.

TABLE V

EFFECTS OF β-ALANINE AND ISETHIONATE ON TAURINE UPTAKE IN RECTAL GLAND SLICES

Taurine uptake (μmol/g WW ± S.E., $n = 4$) and S_i/S_o were determined in rectal gland slices incubated for 180 min in control elasmobranch Ringer containing [^{14}C]taurine (0.2 mM, 0.1 μCi/ml) with or without the addition of 5 mM β-alanine or isethionate.

	Control	β-Alanine	Isethionate
Taurine uptake (μmol/g WW)	0.279 ±0.004	0.089 * ±0.002	0.250 ** ±0.006
S_i/S_o	2.26	0.42	2.05

* $P < 0.005$ and ** $P < 0.01$ compared with control.

2. Role of Na^+

Total substitution of media Na^+ with either Li^+, choline or K^+ resulted in approx. 85% inhibition of taurine uptake per tissue WW (Table II). In all Na^+-free media, the S_i/S_o ratio was markedly diminished. These data suggest that the rate of taurine uptake into rectal gland slices and the subsequent intracellular accumulation are largely Na^+ dependent. A small component of taurine uptake is detectable in Na^+-free media, possibly by diffusion. Addition of 0.5 mM ouabain to standard Na^+-media resulted in 26% inhibition of taurine uptake at 180 min of incubation (Table II). The inhibition with ouabain was less marked than that seen in Na^+-free media, reflecting the slow action of this inhibitor on the intracellular ionic distribution [7]. Nevertheless, the results are indicative of an involvement of the Na^+/K^+-ATPase in intracellular taurine accumulation. Taurine uptake was also examined as a function of media Na^+ concentration (Fig. 3). Variable Li^+ concentrations were used for iso-osmotic Na^+ substitution Media taurine was kept at 0.2 mM and uptake was determined at 180 min of incubation. Linear regression analysis of Eadie-Hofstee plots (Fig. 3) yielded two apparent affinities for Na^+ (K_m values of 12 and 64 mM of Na^+).

3. Role of Cl^-

Table III depicts the effect of Cl^- substitution on taurine uptake. In media containing either gluconate or propionate in exchange for Cl^-, taurine uptake was reduced by approx. 30% at 180 min of incubation. Thus, uptake in rectal gland slices is in part dependent on extracellular Cl^-. However, it appears that external Cl^- may not be an absolute requirement for taurine uptake as is the case with external Na^+. Furthermore, Table IV shows that taurine uptake is not affected by the addition to standard media of the 'loop-active' diuretics (bumetanide, furosemide, piretanide) which are known to inhibit the $Na^+/K^+/2Cl^-$ co-transport system in rectal gland epithelium [10].

TABLE IV

EFFECTS OF DIURETICS AND DIDS ON TAURINE UPTAKE IN RECTAL GLAND SLICES

Slices were incubated for 180 min in control elasmobranch Ringer containing [^{14}C]taurine (0.2 mM, 0.1 μCi/ml) with or without the addition of 0.1 mM bumetanide, furosemide, piretanide, hydrochlorothiazide (HCTZ) or 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS). Data are mean values ± S.E. in μmol/g tissue wet weight (WW). Compared with control uptake, none of the agents added resulted in statistically significant changes.

	Control	Bumetanide	Furosemide	Piretanide	HCTZ	DIDS
Taurine uptake (μmol/g WW)	0.218 ± 0.008	0.232 ± 0.006	0.213 ± 0.007	0.221 ± 0.006	0.200 ± 0.015	0.261 ± 0.007

Table IV also shows that taurine uptake is not inhibited by hydrochlorothiazide which blocks Na^+/Cl^- symport [11] or by the stilbenedisulfonate DIDS which inhibits the anion-exchange carrier. These data suggest that taurine uptake does not utilize any of these ion-carrier systems for entry into cells.

4. Role of competitive inhibitors

In an attempt to identify possible competitive inhibitors of taurine transport, we tested the effects of isethionate and three amino acids (β-alanine, L-glutamate and DL-aspartate) added at a concentration of 5 mM. Taurine uptake was unaffected by the addition of the dicarboxylic α-amino acids, glutamate or aspartate (data not shown). Table V depicts a marked inhibition of taurine uptake by β-alanine, a structurally-related amino acid with a carboxyl group substituted for the sulfonate group. At 180 min of incubation, β-alanine reduces S_i/S_o to 0.42 (Table V). This reduction is comparable to the self-inhibition observed in parallel studies performed with the addition of 5 mM taurine ($S_i/S_o = 0.37$). However, addition of 5 mM isethionate which carries a hydroxyl group instead of the amino group, resulted in only 10% reduction in taurine uptake per tissue WW (Table V). These studies are consistent with the suggestion that β-alanine shares with taurine a Na^+/β-amino acid transport system exhibiting considerable substrate specificity.

5. Kinetics of taurine efflux

The washout of taurine from rectal gland slices was determined in standard (control) media and in a variety of modified media (Fig. 4 and 5). The kinetics of efflux can be described by a three-compartment model. The initial relatively rapid component of taurine efflux most likely corresponds to the flux of the ^{14}C-label from the extracellular tissue space (see below). The size of this space, determined with ^{14}C-PEG, was on average 22% of tissue wet weight (cf. Ref. 6). Given that taurine is accumulated inside the cells against its concentration gradient, the amount of taurine which corresponds to this space must reflect a percentage of total tissue taurine which is smaller than 22%. For the efflux experiments described here, we calculated that on average 19% of total tissue taurine is distributed in the extracellular tissue space after 180 min of [^{14}C]taurine loading. Note that a comparable value can also be obtained by analyzing the kinetics of taurine efflux within the first 4 min of washout; thus, from the highly derived fast rate constant of taurine efflux (0.7 min^{-1}), we calculated [8,9] that approxi-

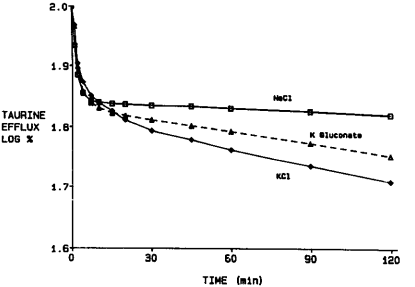

Fig. 4. Effects of media K^+ on taurine efflux from dogfish rectal gland slices. Efflux is given as log (% activity remaining in tissue wet weight) as a function of time. All tissues were first preloaded with [^{14}C]taurine (0.2 mM, 0.2 μCi/ml) in control (standard) Ringer for 180 min; washout was then carried out in one of three media: control Ringer (NaCl, squares); K^+-gluconate Ringer (triangles) where K^+ is substituted for Na^+, and gluconate for Cl^-; and KCl Ringer (diamonds) where K^+ is substituted for Na^+.

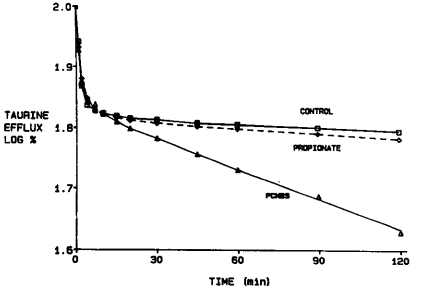

Fig. 5. Effects of media propionate or *p*-chloromercuribenzene sulfonate (PCMBS) on taurine efflux from dogfish rectal gland slices. Efflux is given as log (% activity remaining in tissue wet weight) as a function of time. All tissues were first preloaded with [^{14}C]taurine (0.2 mM, 0.2 μCi/ml) in control Ringer for 180 min. Washout was then carried out in control Ringer without (squares) or with 1 mM PCMBS (triangles), or in propionate media (diamonds) where propionate is substituted for Cl^-.

mately 25% of total tissue taurine corresponds to this kinetic pool. This suggests that the initial component of efflux originates predominantly from the extracellular tissue space. Further support for this suggestion is given by the finding that the profile of taurine efflux curves in the first 4-min-period is not affected by the various modifications of the washout media (Figs. 4 and 5).

The other two components of taurine efflux represent a fast and a slow efflux systems corresponding to the cellular compartment. Fig. 4 compares the washout of taurine in control (NaCl) media and in high K^+-media (K^+-gluconate and KCl). The plotted data were used to calculate the rate constants and the relative pool size of efflux components as described previously [6,8]. In control media, the rate constant for taurine efflux could be determined accurately only for the slow cellular component ($k' = 0.00041 \pm 0.00009$ min^{-1}); the corresponding relative pool size was $65 \pm 3\%$ of total tissue taurine ($n = 6$). From the difference between the size of the extracellular pool and that corresponding to the slow cellular component, the fast efflux component from the cellular pool can be deduced, corresponding to $10 \pm 3\%$ of tissue taurine. As can be seen from Fig. 4, taurine efflux is markedly accelerated in high-K^+ media compared with control. In KCl media, the rate constant k' of the slow cellular component was increased 5.7-fold, to 0.0023 ± 0.0003 min^{-1} ($n = 3$). Here, the fast cellular efflux component could be documented: $k'' = 0.0856 \pm 0.004$ min^{-1}. Taurine efflux is also enhanced in K^+-gluconate media; the rate constant k' of the slow cellular component was increased 4.4-fold, to 0.0018 ± 0.0003 min^{-1} ($n = 3$). The effects of high K^+-media to enhance taurine efflux can be dissociated from cell swelling in the rectal gland. While intracellular water content increases upon incubation in KCl media by approximately 50% (cf. Ref. 7), an actual cell shrinkage (about 10%) takes place in K^+gluconate media (cf. Ref. 12). In Li^+-media, cell water content was increased by approx. 10% while taurine efflux was actually retarded (k' was reduced by 28% compared with control; data not shown). Fig. 5 demonstrates that taurine efflux from slices incubated in propionate media is virtually identical with control despite a 50% increase in cell water content induced by propionate at 120 min of incubation (cf. Ref. 13).

6. Effect of p-chloromercuribenzene sulfonate

Fig. 5 also shows that addition of 1 mM *p*-chloromercuribenzene sulfonate (PCMBS) to

standard media, markedly accelerates taurine efflux; k' increases 7-fold. PCMBS depletes cell taurine content by also inhibiting taurine uptake (0.098 ± 0.005 μmol/g WW vs. 0.199 ± 0.015 in control at 180 min incubation, $n = 4$, $P < 0.001$). The mercurial also induces massive cell swelling; cell water content increases by approx. 60% (cf. Ref. 14).

Discussion

The rate of taurine uptake in rectal gland slices is nearly linear during several hours of incubation in media containing 0.2 mM taurine (close to plasma levels) (Fig. 1). By 180 min of incubation, the average concentration of taurine in cell water is 2-fold that of the media (Tables II–V). Given the relatively high endogenous intracellular concentration of taurine in rectal gland cells of approx. 50 mM [5], this implies that taurine uptake is an active transport process which operates against a steep chemical gradient. The rate of taurine uptake is rather slow; the time required to reach the steady state of intracellular taurine accumulation would take several days. Presumably, the slow uptake can be accounted for by the unfavorable transmembrane taurine gradient in intact cells as well as the low intrinsic permeability of the cell membrane (cf. Ref. 15). The slow efflux of taurine from loaded slices incubated in standard media (Figs. 4 and 5) is also evidence for the relatively low permeability of the cell membrane to taurine transport. The above findings are in agreement with the notion that taurine is an effective intracellular osmolyte which is accumulated in the cell in high concentration against a large gradient.

Taurine uptake in the rectal gland is largely dependent on the concentration of Na^+ in the media (Table II and Fig. 3). To achieve half-maximal uptake into the tissue slices requires an external Na^+ concentration of 31 mM (Fig. 3). It is also likely that the taurine transport system(s) may possess two apparent affinities for Na^+ (Fig. 3) especially in view of the dual affinities for the taurine moiety (Fig. 2). Sodium dependency of taurine transport is in general agreement with results obtained in other tissues of a variety of marine species [2]. The accepted cellular model for active uptake of taurine into cells involves co-transport with Na^+ ions driven by the inwardly-directed Na^+-gradient which is generated and maintained by the activity of the Na^+/K^+-ATPase pump [16]. In the flounder kidney, removal of media Na^+ or ouabain inhibition of the pump decreases taurine uptake [17]. The relatively small effect of ouabain on the taurine uptake by rectal gland slices during 180 min of incubation probably reflects the relatively slow rate constants for the exchange of Na^+ and K^+ in the rectal gland cells [6]. Nevertheless, the data are still consistent with an involvement of the Na^+/K^+-ATPase in intracellular taurine accumulation.

The absence of Cl^- in the medium reduces taurine uptake by approx. 30% (Table III) irrespective of whether the anion is replaced by the relatively impermeant gluconate (which produces slight cell shrinkage) or the more permeant propionate (which results in marked cell swelling) (cf. Ref. 13). These observations suggest that the distinct Cl^- dependence of the taurine uptake is not related either to changes in cell volume, or the modifications of the membrane potential related to the relative permeabilities of the anionic species. Moreover, since 'loop-active' diuretics have no inhibitory effect on taurine uptake (Table IV), it is suggested that taurine is not taken up as an anion of the $Na^+/K^+/2Cl^-$ co-transport system of the rectal gland [10]. The possibility of Cl^- co-transport with taurine by a $2Na^+/Cl^-$/taurine symport as suggested for the erythrocytes of euryhaline fish [18] does not appear to be an obligate step for taurine transport since taurine uptake by rectal gland slices is only modestly decreased by removal of external Cl^-. Similar partial dependency of taurine uptake on media Cl^- is also seen in studies on kidney of aglomerular fish [15], flounder [17] and rat [19]. Presumably, external Cl^- mostly exerts a stimulatory modification on Na^+/taurine co-transport in the rectal gland, as has been proposed for flounder renal epithelium [17].

In agreement with similar findings in other tissues [2,4,18,20], taurine uptake is markedly reduced by the structurally related amino acid β-alanine (Table V), while no inhibition is observed by glutamate and aspartate. It is presumed that taurine and β-alanine share a common Na^+/β-amino acid membrane carrier which is distinct

from other Na^+-dependent transport systems in terms of substrate and inhibitor specificity. The membrane domain in which the Na^+/taurine co-transport carrier is located in epithelial cells (i.e., luminal vs. basolateral) may vary with the function of different epithelia. In general, the carrier is present in the basolateral membrane allowing for taurine uptake from the blood side. In addition, absorptive epithelia such as the mammalian renal proximal tubule [4,21,22] and the flounder intestine [16] are also endowed with luminal (brush-border) taurine transport carriers. In the rectal gland, it is doubtful that taurine uptake takes place at the luminal membrane as this tissue is a pure secretory epithelium. Thus, it is assumed that taurine uptake into rectal gland cells is mostly localized to the basolateral membrane.

Analysis of the kinetics of taurine uptake revealed the presence of a high-affinity, low-capacity transport system as well as a low-affinity, high-capacity system (Table I). A similar dual system has also been previously described in synaptosomal tissue [23]. Under physiological conditions (0.1–0.2 mM taurine in plasma), the high-affinity system appears to be the more important transport pathway. The apparent K_m for taurine uptake via the high-affinity system (60 μM) is within the range described in most other tissues: 40 μM in brush-border membrane vesicles of rat kidney [4], 17 μM in mouse kidney [21], 44 μM in cultured rat hepatocytes [24] and 80 μM in erythrocytes of starry flounder [18]. In contrast, kinetic studies in the winter flounder reveal larger K_m values (i.e., lower affinity) for taurine transport in the renal tubule [17] and the intestinal epithelium [16]. Apparent differences in the affinities of taurine for the transport carrier may reflect variabilities among tissues and/or species, or possibly inherent differences in the experimental techniques. A possible role for differences in the experimental technique is highlighted by studies of taurine transport in rat liver. Here, the apparent K_m for taurine uptake by cultured hepatocytes is 44 μM [24] compared with 174 μM for taurine uptake by membrane vesicles [25].

The efflux of taurine from cells appears to be a passive process along a favorable outwardly-directed gradient, as has previously been indicated by studies on Ehrlich ascites tumor cells [26]. The high concentration of taurine in rectal gland cells (50 mM) suggests that the basal rate of efflux must be slow. This is evident from experiments which examined the kinetics of efflux in slices incubated in standard Ringer's solution (Figs. 4 and 5). A slow efflux is also found in neural tissue [20], in Ehrlich ascites tumor cells [27] and fish red cells [18,28]. In all these studies, taurine efflux can also be stimulated by hypotonic media and this effect is related to cell volume changes. However, cell swelling is no prerequisite for enhanced taurine efflux [28].

In our studies on rectal gland slices, we wished to examine the kinetics of taurine efflux in isotonic media in order to assess the possible role of cations (Na^+ vs. Li^+ vs. K^-), anions (Cl^- vs. gluconate vs. propionate) and iso-osmotic cell volume changes. Taurine efflux from the rectal gland cells is enhanced in high K^+, unaltered in propionate and retarded in Li^+ media. The observed data (Figs. 4 and 5) suggest that the changes in efflux cannot be predicted from the accompanying alterations in the following parameters: absence of media Na^+ and/or Cl^- per se, changes in intracellular electrical potential, or changes in cell volume. For instance, in the Na^+-free incubation media (KCl or Li^+), rectal gland cells are depolarized to nearly the same degree [7,29]; however, taurine efflux is stimulated in high-K^+ media (Fig. 4) and is retarded in Li^+ media. Moreover, cell volume is increased in either media [7] and also in propionate media [13], but taurine efflux remains unaltered in the latter. Thus, cell swelling (or presumed membrane stretching per se) does not necessarily lead to permeabilization of the cytoplasmic membrane. Conversely, and as evidenced by studies with K^+-gluconate, enhanced efflux of taurine can be seen in the absence of any cell swelling. In rectal gland cells, high-K^+ but not high-Li^+ media also increases the efflux of other osmolytes, e.g., *myo*-inositol [29,30] and trimethylamine oxide [31]. Taken together, these observations indicate that the increase in the efflux of taurine and other osmolytes are only related to the presence of a high K^+ concentration in the incubation medium irrespective of whether cell swelling also occurs. It is possible that extracellular K^+ exerts a selective effect on the cell membrane to increase taurine permeability and thus

brings about a rapid dissipation of intracellular taurine accumulation.

The effects of high-K^+ media are associated with structural alterations in cytoskeletal and membrane elements [32]. The cellular mediators that are responsible for stimulation of osmolyte release remain to be elucidated. It is noted, however, that stimulation of taurine efflux in hypotonic media is thought to be induced by humoral mediators; for instance, cell calcium, protein kinase C and/or calmodulin may play a role in modulating taurine efflux in skate erythrocytes [33] and in ascites tumor cells [34] while norepinephrine and cyclic AMP may suppress volume-induced taurine release in erythrocytes of euryhaline fish [18]. Additional studies are required to examine the mediators of K^+-induced taurine release and their structural correlates.

The inhibiting effect of PCMBS on the uptake of taurine, and the enhancement of taurine efflux by this agent, suggest an involvement of membrane sulfhydryl groups in both transport processes (cf. Ref. 14). The inhibition of uptake by the mercurial may, in part, be related to the dissipation of the transcellular Na^+ gradient [14] which is necessary for the coupling to taurine transport. Moreover, PCMBS-induced dissociation of cytoskeletal proteins [35] may result in alterations in membrane permeability and may be responsible for the marked increase in the efflux of taurine from the cells.

Acknowledgements

This work was supported, in part, by NIEHS-SCOR grant (1P30-ES03828) to the Mount Desert Island Biological Laboratory (Dr. Kleinzeller). Dr. Ziyadeh is a recepient of a fellowship from the Measey Foundation and a First Independent Research and Transition Award (1R29-DK-39656-01.

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