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Sodium Cotransport Systems: Cellular, Molecular and Regulatory Aspects

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Summary

The sodium cotransport systems comprise an important group of transport proteins which are involved in the transport of a variety of organic and inorganic solutes across the cellular membrane of animal cells. These systems play a central role in a wide variety of cellular and biochemical processes. We summarize here the current state of knowledge regarding the variety, structure and regulation of this important group of membrane proteins.

Introduction

The cellular membrane, because of its hydrophobic lipid bilayer, acts as a barrier to most polar solvents. Consequently, in order to transport polar molecules across these membranes, specialized selective transport molecules have been evolved. One of the major groups of systems responsible for carrying out these transport processes are the sodium cotransport systems which are responsible for the cotransport of sodium and one of a variety of inorganic or organic solutes (depending on substrate specificity) across the plasma membrane. These systems are widely distributed throughout the animal kingdom and are found in higher animals in a variety of different tissues and cell types. They play an important role not only in the transport of cellular nutrients but also in pH regulation, growth control and also, in certain cell types, transcellular transport. In this review, we will attempt to summarize the information currently available regarding their specificity, their tissue and cellular location, their possible structure and components, and finally the physiological and cellular basis for the regulation of these systems. As most studies with sodium cotransport systems to date have been with those present in the cells of the kidney and small intestine, we will concentrate on the systems present in these tissues. The general principles learned from these sodium cotransport systems, however, are probably applicable to those systems present in other tissues.

Nature of Sodium Cotransport Systems

Sodium cotransport involves the simultaneous transfer of one or more sodium ions and one or more inorganic or organic solutes across the plasma membrane. This transport may be either in opposite directions (antiport), e.g. with such ions as H^+ and Ca^{2+}, or more commonly in the same direction (symport), e.g. as with the cotransport of sugars, amino acids, bile acids, neurotransmitters, and such inorganic ions as phosphate, sulphate, and chloride (see Table 1).

Solute transport against a concentration gradient may be directly (primary active) or indirectly (secondary active) coupled to an exergonic chemical reaction. In mammalian cells, this chemical reaction generally involves the hydrolysis of ATP. Sodium cotransport systems are secondary active transport systems, and are indirectly coupled to the plasma membrane-bound Na^+, K^+-ATPase. This enzyme is responsible for the generation and maintenance of the electrochemical potential difference of sodium concentration, which provides the driving force for the sodium cotransport systems. The transport process itself is accomplished by the movement of sodium down its own electrochemical potential difference, while the cotransported solute is transported against its electrochemical potential difference. The number of sodium ions transported per solute translocated (the stoichiometry of this transport system) determines the electrogenicity of the transport system and the extent to which the sodium gradient and the membrane potential exert their driving forces on the transport system. It is interesting that to date all reported sodium cotransport systems are either electroneutral or electrogenically positive. Thus uptake of anions into a cell via sodium cotransport systems is no longer opposed or is even facilitated by the negative membrane potential.

Certain sodium cotransport systems also require the presence of other ions such as potassium or chloride for their operation. The involvement of additional ions in sodium cotransport has obvious implications not only for the electrogenicity of such systems but also for the net driving force available for transport.[1] Examples of systems requiring the involvement of potassium include the extensively studied $Na^+/K^+/2Cl^-$ cotransporter[2] and the sodium-glutamate cotransport system[3] (see also Table I). In the latter, the potassium gradient provides an additional favourable driving force for the cotransporter. In other cotransport systems, which have additional ion requirements (especially those of the sodium-neurotransmitters[4]), chloride has been shown to be required for activity. The actual role of chloride ions in these systems is not yet clear. However, it is likely that chloride is

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Table I. Sodium cotransport systems

		(*a*) Antiport systems	
Na/H^+	Various tissues and cell types. Also in fibroblast and kidney epithelial cell cultures (LLC-PK_1, MDCK). ?Ubiquitous	Apical	pH regulation ?participation in the initiation of proliferation and differentiation
	Amphimura kidney	Also basal lateral	
Na/Ca^{2+}	Kidney and small intestine brush border membranes (BBM), liver adrenal medulla, pancreas, nerve, skeletal, cardiac, arterial, uterine muscle cells. Various invertebrate systems. LLC-PK_1 cells	Basal lateral	Calcium resorption or secretion. Involved in intracellular Ca^{2+}-regulation. Intracellular stimuli
		(*b*) Symport systems	
Na/phosphate	Kidney, intestine, erythrocytes, cell cultures:– Ehrlich ascites, nerve, LLC-PK_1, mouse fibroblasts, L1210 leukaemia, OK cells	Apical	Resorption. Cellular uptake
Na/sulphate (and thio-sulphate)	Kidney (proximal tubule), intestine, marine teleost proximal cells	Apical	Resorption ?Secretion
Na/iodide	Thyroid cells:– bovine, rat, turtle; in slices and culture	Apical	—
Na/K/2Cl	Kidney (TALH), acinar cells of the pancreas, serous glands of the salivary glands, trachea, erythrocytes, flounder intestine, cultured kidney, fibroblast, SMC, endothelial cells	Apical	Resorption
	Shark rectal gland	Basal lateral	Secretion
Na/sugars			
(*a*) Na/glucose 1:1 stoichiometry	Kidney (pars convoluta of proximal (tubule), BBM of Atlantic hagfish archinephric ducts	Apical	Resorption
(*b*) 2:1 stoichiometry	Kidney (pars recta of proximal tubule), small intestine, *Necturus* and winter flounder and lobster hepato-pancreatic BBM, LLC-PK_1, OK cells and primary proximal tubule cell cultures	Apical	Resorption
(*c*) Na/myo-inositol	Kidney, small intestine	Apical	Resorption
Na/organic anions (carboxylic acids)	Kidney (proximal tubule), small intestine		
(*a*) Monocarboxylic		Apical	Resorption
(*b*) Dicarboxylic and tricarboxylic acid		Apical	Resorption
(*c*) Dicarboxylic acid		Basal lateral	Secretion
Na/bile acids	Liver, hepatocytes, small intestine, kidney	Basal lateral Apical	Secretion Absorption
Na/amino acids			
(*a*) Na/acidic amino acids			
Asp, glu (K^+-dependent)	Kidney and small intestine, central nervous system cells, cultured baby hamster kidney cells, liver	Apical/basal lateral (identical system) Apical	Resorption/ nutrition
Asp, glu (K^+-independent)	Small intestine, liver, turtle and crabfish retina, crab nerve, skin fibroblasts, rat hepatocytes, LLC-PK_1	Apical	Resorption/ nutrition
(*b*) Na^+/basic amino acids (Sys y^+)	Fibroblasts, HTC hepatoma cells	Apical	Resorption
Na^+/arg, lys (ala inhibitable)	Kidney and small intestine BBM	Apical	Resorption
(*c*) Na/neutral amino acid			
A-system (short polar or linear side chains)	Variety of avian and mammalian cell types. Ehrlich ascites cells.	—	Nutrition
	Various fibroblast cell cultures.	Apical	Nutrition
	Small intestine?, kidney?, rat hepatocytes, LLC-PK_1 cells (polarized), MDCK cells	Basal lateral	Nutrition
ASC (ala, ser, cysteine)	Rabbit reticulocytes, rat hepatocytes, pigeon erythrocytes	—	Nutrition
	Hamster ovary cells, human fibroblasts, small intestine?, kidney?, rat hepatocytes, LLC-PK_1 (polarized) cells, MDCK cells	Apical Basal lateral	Nutrition Nutrition
N-System (Gln, asn, his)	Liver, hepatocytes	Apical	Resorption
Specific systems			
	?Kidney and small intestine BBM-specific?	Apical	Resorption

Table I (continued)

Gly (variant of A?)	
Gln	
Cys	
Gly, ala, phe, pro taurine or β-ala	
Ala (A-system) Na/neurotransmitter	*Xenopus laevis* oocyte
γ-aminobutyric a Glu (see acidic amino acids)	Rat brain, insect nervous system
Serotonin (Cl^- and K^+-dependent)	Platelets, red blood cells, rat brain
Norepinephrine (Cl^--dependent)	Rat brain, rat heart
Dopamine $(C)^-$-dependent)	Rat brain

cotransported and a chloride gradient (out-to-in) may also serve as a driving force. Other chloride-requiring non-neurotransmitter sodium cotransport systems have also been reported,[5,6] (see Table I). Although currently few systems have been shown to have additional ion requirements for activity, it may prove that such involvement is more widespread than is currently thought.

Location and Functions of Sodium-Cotransport Systems

Tissue and Cellular Location

As mentioned in the introduction, sodium cotransport systems have been demonstrated in higher organisms in a variety of tissues including endothelium, muscle, brain, thyroid, liver, placenta, salivary gland, pancreas, intestine and kidney. The sodium cotransporters in these tissues have been studied by a variety of techniques including the use of whole organs, tissue slices using micropuncture and electrophysiological methods and more recently with cell culture systems or membrane vesicles derived from defined tissue or cellular locations. These studies have demonstrated the presence of sodium cotransport systems in both epithelial and non-epithelial cells, the cellular cotransporter complement depending on the cellular type and its function. The variety of the different sodium cotransporter systems, their tissue and cellular distribution are shown in Table I. As to the physiological role of the different sodium cotransport systems, it is likely that their functions are not identical in these different cell types. In non-epithelial cells, these systems have been demonstrated to be involved in such varied functions as nutrient uptake, intracellular pH regulation, volume regulation and growth control. In epithelial cells, sodium cotransport systems have been shown in addition to be responsible for transcellular transport.

Subcellular Location and Role in Transcellular Transport

Sodium cotransport systems are asymmetrically situated within the cell membrane, i.e. depending on the cell type and cotransport system, and are inserted either in the apical or basal-lateral membrane surface. It is this asymmetrical distribution together with the nature of this system that makes possible and even determines the direction of transcellular solute flux in epithelial cells, (see Fig. 1*a*). As previously stated, sodium cotransport sys-

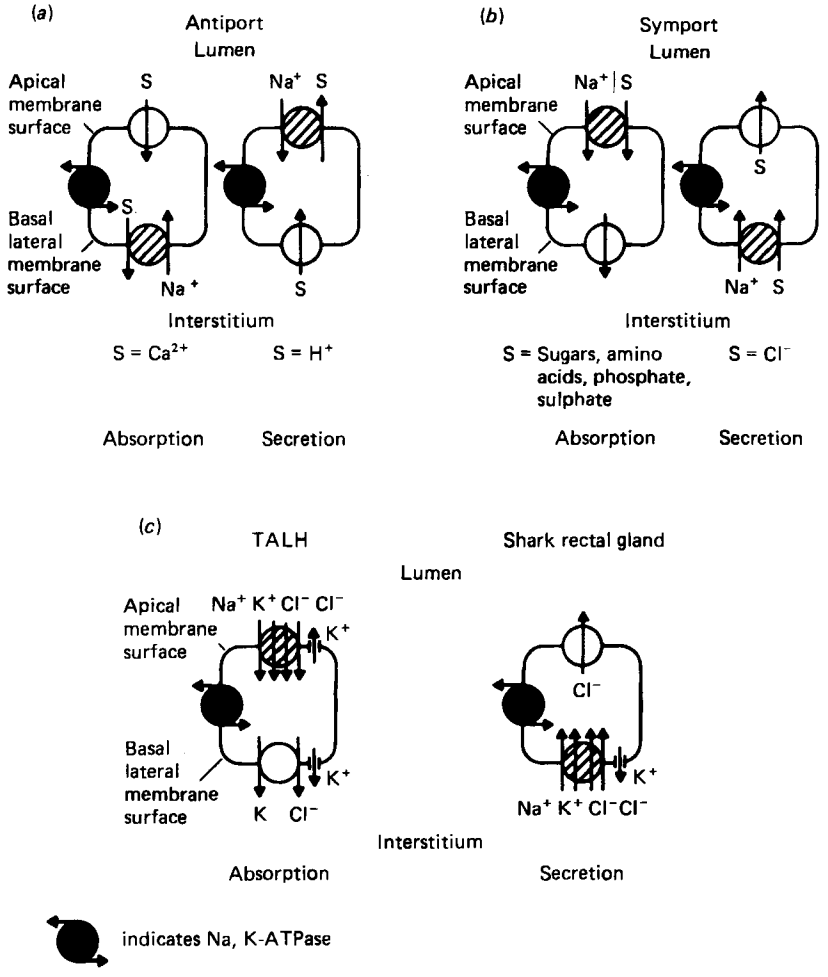

Fig. 1. *Demonstration of the role of sodium cotransport systems in absorption and secretion. (a) Role of antiport systems; (b) Role of symport systems; (c) Demonstration that insertion of a sodium cotransport system, as exemplified by the Na/K/Cl cotransport system, in different membrane surfaces controls the direction of transcellular transport. Thus a system may play a secretory role in one cell type and an absorptive role in another.*

tems can be either symporters or anti-porters, the former facilitating cellular accumulation against a concentration gradient whereas the latter enables removal of solutes against a concentration gradient. Both types of system may, however, play a role in either absorption or secretion, as shown in Fig. 1*a*. Fig. 1*b* also demonstrates that it is possible for a sodium cotransport system to fulfil a different function in different epithelia. It can be seen here that in the thick ascending limb of Henle's loop (TALH) cells the $Na^+/K^+/2Cl^-$ cotransport system participates in active chloride absorption, chloride movement being from the apical to basal lateral surface.[2] In shark rectal gland, however, it is involved in chloride secretion, this movement being from the basal lateral to apical surface.[7]

In all the above situations the location of the Na^+,K^+-ATPase, which provides ultimately the driving force for these systems, plays no role in the determination of the direction of the transcellular solute flux, although in the majority of cell types this enzyme is restricted to the basal lateral membrane surface.

Molecular Structure of Sodium Cotransport Systems

Although many different sodium-dependent cotransport systems have been demonstrated, little is yet known about their structure, and attempts to identify these systems have often produced conflicting or confusing results. The transporter molecule is, however, generally considered to be an intrinsic membrane protein, which can only be removed following disruption of the lipid bilayer. In some instances, evidence has been provided that this molecule is inserted asymmetrically into the membrane.

A variety of different approaches to the identification and isolation of sodium cotransport systems have been applied, and these different procedures have often given vastly different molecular weight estimates of the components of these systems. Many of these approaches have involved negative extraction procedures whereby with the use of proteases and/or detergents most or all proteins other than the transport protein of interest are removed from the membrane. This procedure is then often followed by positive extraction of the system via a variety of separative electrophoretic or chromatographic techniques.[9] These extraction procedures have often been facilitated by the pre-identification or 'labelling' of the transport system of interest, by the use of either (*a*) affinity labelling with substrate analogues; (*b*) radiolabelled specific transporter inhibitor such as bumetanide, or phlorizin, or their derivatives; or (*c*) fluorescent reporter groups (see Table II for examples). Such substrate studies generally involve a two-step procedure. The latter studies have proved of particular interest as addition of substrate molecules (e.g. sodium or glucose for the sodium/glucose transporter)[10] to these 'labelled' molecules has been demonstrated to produce quenching of fluorescence, being indicative of conformational changes in the carrier, as would be expected in the binding of a transporter substrate molecule or during trans-membrane transport of the substrate. A further approach, the considerable potential of which has not yet been fully realized, involves the production and use of monoclonal antibodies against these cotransport systems.[11] Such antibodies provide the possibility not only for identifying these transporters and for isolating their molecular components in large amounts by immuno-affinity chromatography, but also for mapping the functional domains of these systems when a variety of antibodies are employed.

Confirmation as to the identificaiton of these proteins as the putative transporters (or sub-units thereof) can be demonstrated by reconstitution experiments. These experiments involve the insertion of the membrane proteins into liposomes formed with natural or artificial lipids and the subsequent examination of the transporting capabilities of these liposomes.[12]

An alternative approach to the determination of the molecular weights of transport proteins, which unlike the above procedures involves no membrane disruption or protein extraction, is the use of radiation-inactivation procedures using a linear electron accelerator. This technique has indicated a target size (which is considered to be indicative of the molecular weight of the active component of a system) greater than those methods which have involved the identification of transport systems under denaturing (or partially denaturing) conditions. Molecular weight estimates using this technique for the sodium–glucose transporter have, for example, given values of *ca.* 345 kDa,[13] as opposed to values varying from 30 to 70 kDa (under fully denaturating conditions) and 160 kDa under conditions that cannot exclude incomplete dissociation or reaggregation of proteins (see Table II).[14] Such data are consistent with the sodium–glucose cotransport system being a multimeric system composed of a number of polypeptide sub-units that are essential for the transport of glucose and sugar across the membrane. Although very little data are available for radiation-inactivation studies for other systems, values obtained for the $Na^+/K^+/2Cl^-$ cotransporter have also indicated a larger active component for this system than is indicated from isolation and extraction procedures[14]. It is thus likely that other sodium-cotransport systems are also multimeric systems composed of identical or non-identical polypeptide components.

Regulation of Sodium-Cotransport Systems

Many of the earlier studies on the regulation of sodium cotransport systems were carried out with either intact animals, organs or tissue fragments. Consequently, care must be taken in the interpretation of such experiments since certain treatments or exogenous administration of hormones may cause the release of other hormones or growth factors. The development of hormonally responsive cell cultures has, however, greatly facilitated such studies by enabling the study of specific systems under fully defined conditions. Thus, it has been feasible to study directly the actual cellular mechanisms involved in transport system regulation. However, care must also be taken in the extrapolation of these results to the whole organism.

Sodium cotransport systems have been shown to be responsive to a variety of conditions including either high concentrations of or deprivation of substrate or treatment with a variety of hormones and growth factors. These responses may be either stimulatory or inhibitory depending on the system and stimuli. These regulatory responses can be conveniently divided into two main groups, those which exhibit a rapid response (often within minutes and generally within 1–2 h) and those which produce a measurable effect only after 12–24 h or more.

Inducers of the rapid response are generally hormones or growth factors (see Table III), whose action is commonly mediated by secondary messengers and various intracellular regulatory pathways. These rapid responses generally involve an alteration of the V_{max} without an alteration of the K_m of the

stimulated system. It is likely that these effects involve no *de novo* protein synthesis, as these responses are unaffected by inhibitors of transcription or translocation. Two main mechanisms of action have been proposed to explain these rapid responses: (*a*) an *in situ* modification of existing cotransporter sites (or cotransporter regulator molecules), and/or (*b*) a 'shuttling mechanism' between active and inactive transport sites. A number of possible mechanisms of action for *in situ* modifications of co-transporters have been postulated. These have included phosphorylation via any one of a number of processes involving cyclic AMP, tyrosine specific kinase, or protein kinase C (whether direct or through diacylglycerol, following stimulation of phosphoinositide phosphodiesterases). Other mechanisms suggested have included NAD-dependent ADP-riboxylation, Schiff base formation, or a decrease in the rate of protein dephosphorylation. A rapid membrane recycling process has been postulated for the 'shuttling mechanism' between an active cellular membrane pool and an inactive reservoir in cytoplasmic vesicles, the relative proportions of which are altered following stimulation. A precedent for such a recycling mechanism in transport processes comes from studies involving the H^+-pump in the renal tubule[15] and sodium-independent glucose transporter in adipocytes[16]. Phosphorylation or NAD-dependent ADP-ribosylation could also play a role in a 'shuttling process' by inducing an alteration in cotransporter recycling, thus altering the rate of insertion into the plasma membrane or cytoplasmic 'depot vesicles'. Another hypothetical possibility (for which there is currently no available evidence) is the modification of the transporter in the cytoplasmic pool prior to insertion into the surface membrane. In addition to such hypotheses, a further mechanism that has been postulated to play a role in sodium-dependent cotransport regulation (in view of the dependence of these systems on the flow of sodium ions) has been one involving changes in the transmembrane electrochemical gradient of sodium. Although there are a number of hormones that rapidly induce such changes, it is difficult to explain the specificity of transport responses observed by such a mechanism.

Long-term or 'adaptive' responses are induced by a variety of stimuli including hormones, such as glucocorticoids, thyroid hormones and vitamin D, and certain metabolic stimuli, such as metabolic acidosis or certain dietary alterations (see Table 3). These responses may result in alterations in the V_{max} and/or K_m of the transporters and involve *de novo* protein synthesis, as demonstrated by the inhibition of response by inhibitors of translation or transcription. It is thought that these 'adaptive' responses bring about an induction of transcription and translation (via either a primary or a secondary response) resulting directly in the production of either cotransporter proteins and/or modifying enzymes of these systems. Alterations in the V_{max} can thus be explained in terms of an increased number of functionally active cotransporters in the membrane, the direct result of either increased synthesis or decreased degradation. Changes in the K_m of a system, however, could involve changes in the synthesis

Table II. Structure and mode of identification of sodium cotransport systems

System	Size of component (kDa)	Mode of identification
Na/phosphate	3 (proteolipid)	Phosphate binding, chromatographic separation of product, cAMP-dependent
	62	phosphorylation of membrane suspensions of kidney
Na/bile acids	34, 37, 50, 54	Labelling of hepatocyte membrane with a DIDS derivative
	34, 37 48–50, 52–54	Photoaffinity labelling of rat liver membranes with a somatostatin derivative
Na/organic acids	26, 52, 65, 108	Photoaffinity labelling of basal lateral membranes of proximal tubule
Na/neurotransmitter (GABA)	24	Subfractionation of detergent-solubilized fractions into liposomes and examination of GABA uptake
Na/K/2Cl	34	Photolabelling with [^{3}H] bumetanide of TALH (apical membrane)
	90	Radiation inactivation of microsomes derived from outer renal medulla
	170	Photolabelling (blocked by bumetanide) with a benzophenone derivatives of bumetanide. A 34 kDa protein was incompletely blocked
Na/D-glucose (kidney)	2 × 85 units	Rabbit renal BBM
	60–70 80, 2 × 74, 45	Renal cortex membranes covalently radiolabelled with phlorizin or glucose analogues
		Radiation-inactivation studies with BBM vesicles
	345	D-glucose transporting component
	230	Phlorizin binding component
(Small intestine)		
	57, 49	Subfractionation into liposomes
	72	Subfractionation into liposomes
	72	Monoclonal antibody labelling of BBM proteins
	70–71	FITC labelling of cotransporter site following general labelling of other proteins with non-fluorescent PITC
BBM subfractionation into liposomes and subsequent examination by:		
	160	SDS gel separation

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Table III. Regulation of sodium-cotransport systems

	Rapid effects*		Inductive (long-term) effects†	
System	Inducer	Effect	Inducer	Effect
(*a*) Na/phosphate				
Kidney and intestine brush-border membranes	Parathormone, insulin, calcitonin, growth hormone, high phosphate, nicotinamide, atrial natriuretic hormone, plasma CO_2	Typically a V_{max} change, cyclic AMP, calcium-dependent. Protein kinase and phosphorylation involved in response	Thyroid hormones, glucocorticoids, vitamin D, metabolic acidosis, phosphate deprivations	Increased uptake Alteration of V_{max} and/or K_m
LLC-PK_1	Low phosphate		Increased activity	
(*b*) Na/iodide				
Bovine, turtle thyroid			Thyroid stimulating hormone	Increased uptake, probably V_{max} increase
(*c*) Na/Calcium				
Rat renal cortex	Parathormone, cyclic nucleotides, forskolin, may be activated by ATP	Increased V_{max}, or K_m and V_{max}.	—	—
Heart plasma membranes		?involves phosphorylation. K_m and V_{max} increased, involves phosphorylation, requires Ca^{2+} and calmodulin		
(*d*) Na/K/2Cl				
Avian erythrocytes, shark rectal glands	cAMP and cGMP Ca^{2+}	Stimulation Inhibition	— —	— —
Flounder intestine	cAMP and cGMP	Inhibition	—	–
cultured smooth muscle cells (SMC)				
Fibroblasts, SMC in culture	Various:– including serum, EGF, insulin, arginine, vasopressin, FGF, thrombin	Stimulation	—	—
TALH cell cultures			Cell density, time post-confluence	Increase followed by decrease
Mouse kidney medulla	Antidiuretic hormone	Stimulation, increase in V_{max}	—	—
Various tissues and cells (MDCK cells)	ATP requirement	Phosphorylation of transporter?	—	—
(*e*) Na/amino acids				
(i) N-system				
Cultured hepatocytes	—	—	Amino-acid deprivation (prevented by his plus certain non-substrate amino-acids)	Stimulation, V_{max} increase
(ii) A-system				
Various tissue and cell cultures	—	—	Amino-acid starvation	Stimulation, V_{max} increase
Hepatocytes	Glucagon	Stimulation increase in V_{max}	Glucagon	Second phase of glucagon effect. Increase in V_{max}, involves TIP synthesis
Various cell cultures, including fibroblasts, MDCK cells, etc.	Various growth factors e.g. EGF, FGF, PDGF, NGF, MSA, cAMP	Generally increased V_{max}	Various hormones, e.g. insulin, glucocorticoids, PTH, androgens, oestrogens, catecholamines	May alter V_{max} and or K_m
(*f*) Na/D-glucose				
LLC-PK_1	—	—	Cell density, time post-confluence (maturation)	Increase in uptake, correlated with transport in apical membrane microvilli
LLC-PK_1	—	—	Low glucose concentration	Increased activity
			High glucose concentration	Reduced activity (correlated with number of transport sites)
Intestinal brush border	—	—	High glucose concentration	High activity
			Low glucose concentration	Low activity
(*g*) Na^+/H^+				
Various cell cultures: fibroblasts, SMC, myoblasts, glioma, LLC-PK_1	Serum, EGF, PDGF, insulin, vasopressin, angiotensin, phorbol esters	Stimulation. Generally increased V_{max}	—	—

Table III (continued)

Platelets, fibroblasts	Ca^{2+} ionophores, thrombin	Stimulation Generally increased V_{max}	—	—
Lymphocytes	Lectins	Generally increased V_{max}	—	—
Erythrocytes, thymocytes, muscle, kidney cells	Osmotic shrinking	Generally increased V_{max}	—	—
Renal brush-border vesicles, *Necturus* gall-bladder epithelium	cAMP, PTH	Inhibition	—	—
Kidney–proximal tubule cells	—	—	Long-term exposure to acidosis. Thyroxine, gluco-corticoids	Stimulation Generally increased V_{max}

of enzymes involved in the processing of the cotransporter, thus producing a molecule with altered properties. Changes in both the V_{max} and K_m could in principle involve a combination of the production of both cotransporter proteins and 'modifying enzymes'.

It should also be noted that additional control processes may be superimposed upon these two major regulatory control types. This is clearly demonstrated by the repression (reversal) of increased uptake of substrate amino acids of the A system (for neutral amino acids), following induction by amino acid starvation of glycogen-pretreated rat hepatocytes.[17] This repression is similar to the inductive response in that it involves both mRNA and protein synthesis. This process appears to involve the synthesis of a protein termed Transport Inactivating Protein (TIP) which is coded for by mRNA which, like histone DNA, appears not to contain any poly(A) sequences. These studies have led the authors of these studies to postulate that TIP is a nucleo-regulatory protein that modifies the transcription of System A transporter proteins. The repressor amino acids are thought to maintain sufficient levels of TIP via an apo-activator protein, which leads to increased transcription of the TIP gene and hence, system A repression.[18]

Future Research on Sodium Cotransport Systems

As with many other systems, studies with sodium cotransport systems are entering into an exciting new era with the availability of a variety of modern cell culture, monoclonal antibody and recombinant DNA techniques.

Current technologies now enable the establishment or 'manufacture' of epithelial cell lines of defined cellular and tissue origin, which are capable of the maintenance of their cell-type specific characteristics (which may include specific sodium-cotransporters) without transformation by intact virus particles. This has already been achieved with kidney cells of defined cellular origin (the TALH) by the introduction of cellular or viral oncogenes[19] and such a process has obvious potential for the 'immortalization' of other differentiated cell types. Such cell lines should enable a clarification as to which cells are capable of the expression of the different sodium cotransporter systems and facilitate the study of the cell biology of these systems. Analysis of such differentiated cell lines together with the use of defined serum-free medium should permit the identification of the factors controlling the activity of the sodium-dependent cotransport systems and eventually their modes of control at the molecular level. Cell lines of indefinite growth capabilities should also be amenable to a genetic approach to the study of cotransport systems; it should be possible to isolate mutants that are defective in these systems,[20] providing additional information as to their structure and modes of regulation. Thus it should be possible not only to determine the distinct steps in the transport process, the mechanism of the observed transport system responses and stimuli-induced metabolic pathways, but also to map the genes coding for and regulating these systems.

The development of procedures for the preparation of monoclonal antibodies has also permitted the production of antibodies against distinct cell-type-specific surface membrane antigens, including sodium cotransporters.[10] Such antibodies provide the potential for a variety of avenues of research, such as the identification of the protein components of these systems, the mapping of their different structural and functional domains (i.e. cytoplasmic, membrane and active site), and the potential isolation of these transport systems in relatively large amounts by the use of immunoaffinity chromatography.

The combination of the use of differentiated cell lines (or their genetically manipulated derivatives) for the preparation of cDNA libraries, enriched for sodium cotransporters, together with monoclonal antibodies that recognize these systems, should provide a powerful tool for the study of the various systems. Thus it should be possible in the near future to determine the precise molecular structure of a variety of sodium cotransport systems, as has already been successfully carried out with the sodium-independent D-glucose cotransporter of the erythrocyte.[21] Comparative analyses of the primary sequences of different sodium cotransporters, and even of the sodium-independent transport systems, should also provide information as to whether there are possible evolutionary relationships between these transporters. That is, whether the different systems are structurally related and derived from common ancestoral genes. Re-expression of these genes following their introduction, either in their wild-type form or as *in vitro* mutated genomic or complementary DNA, into cells that normally do not express these genes, should also provide information on the control of expression of these genes and the sequences which are important in the activation of these systems.

Thus, in the very near future, we can look forward to a breakthrough in our understanding of the structure, regulation and possible evolutionary relationships between the variety of the different sodium cotransport systems.

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Pharmacogenetics of Methyl Conjugation and Thiopurine Drug Toxicity

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Summary

Pharmacogenetics is the study of inherited variations in drug response.[1] *Pharmacogenetics uses the techniques of pharmacology, population genetics, biochemical genetics and, most recently, molecular biology, to study the biological basis for individual variation in therapeutic response and in the occurrence of adverse reactions to medications. Most pharmacogenetic experiments deal with inherited differences in drug metabolism. The discussion here will review inherited variation in the activity of thiopurine methyltransferase, an enzyme that catalyzes the methyl conjugation of an important group of drugs, the thiopurines.*

Introduction

Methylation is an important pathway in the biotransformation of many drugs, neurotransmitters and xenobiotic compounds.[2] It was over a century ago that Wilhelm His first described the methyl conjugation of an exogenous compound, pyridine.[3] Since that time many drug metabolizing methyltransferase enzymes have been described. S-Adenosyl-L-methionine is the methyl donor for most of these enzymes.[2] Until recently, factors responsible for individual variations in methyl conjugation in humans were not understood. However,