ACCELERATED PUBLICATION

Human sodium-coupled citrate transporter, the orthologue of *Drosophila* Indy, as a novel target for lithium actionKatsuhisa INOUE*, Lina ZHUANG*, Dennis M. MADDOX†, Sylvia B. SMITH† and Vadivel GANAPATHY*¹

*Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, GA 30912, U.S.A., and †Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta, GA 30912, U.S.A.

NaCT (sodium-coupled citrate transporter) is an Na⁺-coupled citrate transporter identified recently in mammals that mediates the cellular uptake of citrate. It is expressed predominantly in the liver. NaCT is structurally and functionally related to the product of the *Indy* (I'm not dead yet) gene in *Drosophila*, the dysfunction of which leads to lifespan extension. Here, we show that NaCT mediates the utilization of extracellular citrate for fat synthesis in human liver cells, and that the process is stimulated by lithium. The transport function of NaCT is enhanced by lithium at concentrations found in humans treated with lithium for bipolar disorders. Valproate and carbamazepine, two other drugs that are used for the treatment of bipolar disorder, do not affect the function of NaCT. The stimulatory effect of Li⁺ is specific for human NaCT, since NaCTs from other animal species are either inhibited or unaffected by Li⁺. The data also suggest that two of

the four Na⁺-binding sites in human NaCT may become occupied by Li⁺ to produce the stimulatory effect. The stimulation of NaCT in humans by lithium at therapeutically relevant concentrations has potential clinical implications. We also show here that a single base mutation in codon-500 (TTT → CTT) in the human NaCT gene, leading to the replacement of phenylalanine with leucine, stimulates the transport function and abolishes the stimulatory effect of lithium. This raises the possibility that genetic mutations in humans may lead to alterations in the constitutive activity of the transporter, with associated clinical consequences.

Key words: cholesterol synthesis, *Indy*, lipid synthesis, lithium therapy, mood stabilizer, obesity, sodium-coupled citrate transporter.

INTRODUCTION

The *Indy* (I'm not dead yet) gene in *Drosophila* codes for a plasma membrane transporter that transports various dicarboxylates, such as succinate, as well as the tricarboxylate citrate [1,2]. Dysfunction of this gene leads to lifespan extension in this organism [3]. During our search for the mammalian orthologue of *Drosophila* *Indy*, we have recently identified NaCT (sodium-coupled citrate transporter) in rat and human tissues as a plasma membrane transporter, with functional characteristics similar to those of *Indy* [4,5]. NaCT belongs to the sodium dicarboxylate/sulphate co-transporter gene family (*SLC13*) [6]. Whereas the previously identified mammalian NaDCs (sodium dicarboxylate co-transporters) NaDC1 and NaDC3 recognize only dicarboxylates as substrates, the newly identified NaCT accepts various dicarboxylates as well as the tricarboxylate citrate as substrates. In fact, human NaCT transports citrate much more effectively than succinate. The Na⁺:substrate stoichiometry for NaCT is 4:1, irrespective of whether the transported substrate is a dicarboxylate or a tricarboxylate (K. Inoue, Y. J. Fei and V. Ganapathy, unpublished work). In contrast, the Na⁺:substrate stoichiometry for NaDC1 and NaDC3 is 3:1 [6]. Earlier studies have shown that NaDC1 and NaDC3 are inhibited by lithium [6,7]. Urinary excretion of dicarboxylates is increased significantly in patients undergoing lithium therapy for affective disorders [8], and inhibition of NaDC1 in the kidney by Li⁺ is believed to be the cause for this phenomenon [9]. Whether there are any consequences of Li⁺-induced inhibition of NaDC3 during lithium therapy is not known. Since NaCT is structurally and functionally similar to NaDCs, we asked whether

the transport function of NaCT is also inhibited by lithium. These studies have led to unexpected findings. Whereas NaCTs from non-human organisms are either inhibited or unaffected by Li⁺, human NaCT is markedly stimulated by Li⁺. These studies identify human NaCT as a novel target for lithium action, and have potential clinical implications for individuals who are on lithium therapy for affective disorders. We also show here that NaCT facilitates the utilization of extracellular citrate for lipid synthesis in human liver cells, and that Li⁺ stimulates this process. Furthermore, using rat/human chimaeric NaCTs and site-directed mutagenesis, we demonstrate here that a single base mutation in codon-500 (TTT → CTT) in human NaCT, leading to the replacement of phenylalanine with leucine, stimulates the transport function and abolishes the stimulatory effect of lithium. This raises the possibility that genetic mutations in humans may lead to alterations in the constitutive activity of the transporter, with associated clinical consequences.

MATERIALS AND METHODS

Heterologous expression of NaCTs

NaCT cDNAs, cloned from the human hepatocarcinoma cell line HepG2, rat brain, mouse liver, whole zebrafish and whole *Caenorhabditis elegans*, were expressed functionally in an HRPE (human retinal pigment epithelial) cell line using the vaccinia virus expression technique, as described previously [4,5]. The same approach was also used to analyse the function of chimaeric and mutant NaCTs. The transport function of NaCTs

Abbreviations used: *Indy*, I'm not dead yet; NaCT, sodium-coupled citrate transporter; NaDC, sodium dicarboxylate co-transporter.

¹ To whom correspondence should be addressed (e-mail vganapat@mail.mcg.edu).

was monitored by measuring the uptake of [^{14}C]citrate (Moravek Biochemicals, Brea, CA, U.S.A.), using uptake medium containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl_2 , 0.8 mM MgSO_4 and 5 mM glucose, buffered with 25 mM Hepes/Tris, pH 7.5. Uptake measurements were made in parallel in cDNA-transfected cells, and in cells transfected with vector alone. The uptake in vector-transfected cells (endogenous activity) was subtracted from the uptake in cDNA-transfected cells to determine the cDNA-specific activity. When the effect of Li^+ was studied, the uptake medium contained either LiCl or equimolar concentrations of *N*-methyl-D-glucamine chloride. The Na^+ -activation kinetics were analysed by measuring the uptake of citrate at increasing concentrations of Na^+ , with the concentration of *N*-methyl-D-glucamine chloride adjusted appropriately to maintain the osmolality of the uptake medium. The Hill coefficient (*h*; the number of Na^+ ions involved in the activation process) was determined by fitting the data to the Hill equation. A 30 min incubation was used in uptake measurements, since these experimental conditions have been shown to be suitable for measurement of initial uptake rates for NaCTs [4,5].

Uptake measurement in human liver cell lines

The human liver cell lines (HepG2 and Huh-7) were cultured to confluence in 24-well culture plates using an appropriate culture medium. The HepG2 cell line was obtained from American Type Culture Collection (A.T.C.C.; Manassas, VA, U.S.A.). The Huh-7 cell line [10] was provided by Dr Barrie P. Bode (St Louis University, St Louis, MO, U.S.A.). Uptake measurements in monolayer cultures of these cells were measured as described above for the heterologously expressed NaCTs.

Measurement of incorporation of citrate and acetate into cellular lipids

Monolayer cultures of HepG2 cells were incubated with [^{14}C]citrate (0.1 μCi) or [^{14}C]acetate (0.1 μCi) (American Radio-labeled Chemicals, St Louis, MO, U.S.A.) for 24 h in an NaCl-containing medium in the presence or absence of 2 or 10 mM Li^+ . The cellular lipids were then extracted with *n*-hexane/propan-2-ol (3:2, v/v) as described by Scharnagl et al. [11]. The radioactivity associated with the lipid fraction was quantified to calculate the extent of incorporation of extracellularly added [^{14}C]citrate or [^{14}C]acetate into lipids.

RESULTS AND DISCUSSION

First, we tested the influence of Li^+ on the transport function of rat NaCT. These studies showed that the activity was indeed inhibited by Li^+ (Figure 1). However, when we tested the effect of Li^+ on the activity of human NaCT, the results were quite unexpected. The transport activity of human NaCT was not inhibited, but stimulated, by Li^+ (Figure 1). Li^+ caused marked inhibition of citrate uptake via rat NaCT, with an IC_{50} of 2.1 ± 0.8 mM. In contrast, Li^+ stimulated citrate uptake via human NaCT, and the concentration of Li^+ needed for half-maximal stimulation was 2.1 ± 0.7 mM. The plasma concentrations of Li^+ in patients treated with lithium are in the range 0.8–2 mM [12]. At a concentration of 2.5 mM, Li^+ stimulated human NaCT activity 2.4-fold. The stimulatory effect of Li^+ is associated with an increase in substrate affinity, as well as with a decrease in maximal velocity (Figure 2A). In the presence of 10 mM Li^+ , the affinity for citrate increased approx. 7-fold [K_t (the Michaelis-Menten constant for a transport process) in the absence of Li^+ , 702 ± 55 μM ; K_t in the presence of Li^+ , 95 ± 15 μM]. The normal

Figure 1 Differential effect of Li^+ on the uptake of citrate (20 μM) via rat and human NaCT

Shown are data for rat (●) and human (○) NaCTs.

Figure 2 Substrate saturation kinetics and Na^+ -activation kinetics for human NaCT

Shown are the substrate saturation kinetics (A) for human NaCT in the absence (■) and presence (□) of 10 mM Li^+ , and the Na^+ -activation kinetics (B) for human NaCT in the absence (■) and presence (□) of 10 mM Li^+ .

concentrations of citrate in human blood are in the range 100–150 μM [13]. Therefore, under physiological conditions, the increase in substrate affinity is the primary effect of Li^+ on cellular entry of citrate via NaCT. Li^+ cannot support NaCT function in the absence of Na^+ . The stimulation of human NaCT function by Li^+ was observed only in the presence of Na^+ . The stimulatory effect was greater at lower concentrations of Na^+ , and the effect decreased as the concentration of Na^+ increased (Figure 2B). The stimulation was 3-fold at 140 mM Na^+ , but the stimulation was 7-fold in the presence of 60 mM Na^+ . We also analysed the Na^+ :citrate stoichiometry for the transport process in the presence and absence of Li^+ by fitting the data from the Na^+ -activation kinetics to the Hill equation. The Hill coefficient (*h*), which represents the number of Na^+ ions involved in the activation process, was 4.5 ± 0.7 in the absence of Li^+ . This value changed to

Table 1 Differential effects of Li^+ , valproate and carbamazepine on NaCT from different species and on NaCT in human liver cell lines

Values in parentheses represent the percentage of corresponding control value. ND, not determined.

	Control	In the presence of . . .	Citrate uptake*		
			Li^+ (2 mM)	Valproate (0.6 μM)	Carbamazepine (50 μM)
Species of NaCT					
Human	51.8 $\pm$ 2.5 (100)		105.2 $\pm$ 11.6 (203)	57.3 $\pm$ 2.6 (111)	54.1 $\pm$ 2.6 (105)
Rat	148.8 $\pm$ 7.2 (100)		92.3 $\pm$ 8.4 (62)	142.4 $\pm$ 6.3 (96)	133.5 $\pm$ 0.7 (90)
Mouse	42.5 $\pm$ 2.3 (100)		29.4 $\pm$ 1.7 (69)	42.5 $\pm$ 4.4 (100)	43.7 $\pm$ 2.0 (103)
<i>C. elegans</i>	43.5 $\pm$ 2.1 (100)		43.6 $\pm$ 0.8 (100)	46.0 $\pm$ 3.6 (106)	45.1 $\pm$ 1.5 (104)
Zebrafish	485.8 $\pm$ 20.6 (100)		298.0 $\pm$ 12.6 (61)	453.0 $\pm$ 26.6 (93.2)	441.0 $\pm$ 2.7 (91)
Human liver cell line					
HepG2	9.5 $\pm$ 0.3 (100)		21.2 $\pm$ 0.6 (222)	9.9 $\pm$ 0.3 (105)	10.0 $\pm$ 0.2 (106)
Huh-7	10.2 $\pm$ 0.2 (100)		20.0 $\pm$ 0.5 (196)	ND	ND

* The citrate uptake was measured in units of pmol of citrate/ 10^6 cells per min for NaCT from the different species (human, rat, mouse, *C. elegans* and zebrafish); the units were pmol of citrate/min per mg of protein for the human liver cell lines (HepG2 and Huh-7 cells).

1.8 $\pm$ 0.1 in the presence of 10 mM Li^+ . Measurements of citrate transfer and charge transfer in *Xenopus laevis* oocytes expressing mammalian NaCT have shown that the Na^+ :citrate stoichiometry for NaCT is 4:1 (K. Inoue, Y. J. Fei and V. Ganapathy, unpublished work), and the Hill coefficient of 4.5 ± 0.7 determined in the present study is close to the actual value determined in oocytes. Interestingly, the Na^+ :citrate stoichiometry changes to 2:1 in the presence of Li^+ . These data suggest that two of the four Na^+ -binding sites in human NaCT may become occupied by Li^+ during the stimulatory process.

These data show that there is a species-specific influence of Li^+ on the activity of NaCT. To analyse this species-specific phenomenon in a broader scope, we cloned the NaCT orthologues from three additional species: mouse, zebrafish and *C. elegans*. NaCTs from these species also mediate Na^+ -coupled transport of citrate. We then tested the influence of Li^+ on the function of these NaCTs (Table 1). The transport function of mouse and zebrafish NaCTs was inhibited by Li^+ , as was rat NaCT. In contrast, NaCT from *C. elegans* was not affected by Li^+ . To confirm the stimulatory effect of Li^+ on human NaCT that was seen with the cloned transporter, we studied the effect of Li^+ on constitutively expressed NaCT in human liver cells. For this purpose, we used two different human hepatoma cell lines (HepG2 and Huh-7). In both cell lines, the constitutive expression of NaCT was detectable as measured by Na^+ -coupled uptake of citrate. Li^+ stimulated this citrate uptake activity in both cell lines (Table 1).

In addition to lithium, two other drugs, valproate and carbamazepine, are currently used for an effective treatment of bipolar disorder. The activity of human NaCT was not affected by these two drugs at therapeutically relevant concentrations (valproate, 0.6 mM; carbamazepine, 50 μM) (Table 1). The therapeutic plasma levels for valproate and carbamazepine in humans are in the ranges 0.3–0.6 mM and 20–50 μM respectively [14]. Therefore the concentrations of valproate and carbamazepine used in the present study are clinically relevant. The lack of effect of valproate and carbamazepine on human NaCT was also evident with constitutively expressed NaCT in HepG2 cells. These drugs had no effect, not only on human NaCT, but also on NaCTs cloned from other species (Table 1). Thus among the three widely used mood-stabilizers, only lithium has the stimulatory effect on human NaCT.

NaCT is the first plasma membrane transporter identified in mammals that shows a preference for citrate as a substrate, and operates very effectively under physiological concentrations of citrate in the circulation. Citrate has wide biological functions.

It is not only an intermediate in the citric-acid cycle, and hence a critical substrate for energy production, but is also a source of acetyl-CoA in the cytoplasm for the synthesis of cholesterol and fatty acids. Citrate levels in the cytoplasm also signal the energy status of the cell by serving as a potent inhibitor of phosphofructokinase-1, the rate-limiting enzyme in glycolysis. Since the concentrations of citrate in blood are quite high, the presence of a plasma membrane transporter for citrate will provide the cells with a continuous supply of an additional source of citrate for utilization in metabolic pathways. Therefore the recent identification of NaCT as a plasma-membrane, energy-coupled 'uphill' transporter for citrate in mammals is of great biological relevance. The functional characteristics and the tissue distribution pattern of NaCT support a critical role for this transporter in cellular metabolism. The affinity of human NaCT for citrate is ideal for its role in mediating the cellular entry of citrate from blood (K_t approx. 700 μM ; citrate levels in human blood, approx. 135 μM). The transporter is expressed predominantly in the liver. It is also expressed in the testis and brain, but at much lower levels. Liver is metabolically very active, especially in the synthesis of cholesterol and fatty acids and in glucose homeostasis, the biochemical pathways in which citrate has a critical role. *In situ* hybridization studies show that NaCT mRNA is expressed uniformly in perivenous hepatocytes, as well as in periportal hepatocytes (results not shown). These findings are of interest, considering the metabolic role of citrate in fatty acid synthesis, cholesterol synthesis, glycolysis and gluconeogenesis. Citrate is not only a precursor for the synthesis of fatty acids and cholesterol, but is also a potent inhibitor of phosphofructokinase-1, one of the rate-limiting enzymes in the glycolytic pathway. Therefore citrate is an inhibitor of glycolysis, and a stimulator of gluconeogenesis. These four metabolic processes, namely fatty acid synthesis, cholesterol synthesis, glycolysis and gluconeogenesis, exhibit zonal heterogeneity in the liver. Fatty acid synthesis and glycolysis occur predominantly in the perivenous hepatocytes, whereas cholesterol synthesis and gluconeogenesis occur predominantly in the periportal hepatocytes [15]. The observation that NaCT is expressed in perivenous, as well as periportal, hepatocytes suggests a role for this transporter in all of these metabolic processes, irrespective of the zone-specific occurrence of these processes in the liver.

If NaCT has a role in facilitating the hepatic utilization of extracellular citrate for the synthesis of fatty acids and cholesterol, the results of the present study would suggest that the incorporation of extracellular citrate into lipids would be enhanced by

Figure 3 Incorporation of citrate and acetate into lipids in HepG2 cells in the absence or presence of Li⁺

The left-hand histogram shows the incorporation of [¹⁴C]citrate into lipids in HepG2 cells in the absence of Li⁺, or in the presence of 2 or 10 mM Li⁺. The right-hand histogram shows the incorporation of [¹⁴C]acetate into lipids for the same concentrations of Li⁺.

Li⁺ in human liver cells. To test this, we monitored the incorporation of [¹⁴C]citrate from extracellular medium into the lipid fraction that is extractable with *n*-hexane/propan-2-ol in HepG2 cells, and assessed the influence of Li⁺ on the process. The extracted lipid fraction contains triacylglycerols, cholesterol esters and cholesterol. These studies have shown that Li⁺ markedly enhances the incorporation of citrate into this lipid fraction (Figure 3). The stimulation was 2.6 ± 0.1 -fold at therapeutically relevant concentrations of Li⁺ (2 mM). There are additional targets for Li⁺ in mammalian cells. Inositol monophosphatase, inositol polyphosphatase and glycogen synthase kinase-3 β are considered to be the primary targets for lithium action related to the therapeutic efficacy of lithium in the treatment of bipolar disorder [14,16–18]. Of these, glycogen synthase kinase-3 β is involved in hepatic energy metabolism. Therefore it is important to establish that the observed Li⁺-induced increase in the incorporation of extracellular citrate into lipids does not occur through targets other than NaCT. To provide evidence for the involvement of NaCT in this process, we monitored the incorporation of extracellular [¹⁴C]acetate into lipids in HepG2 cells and assessed the influence of Li⁺ on this process (Figure 3). NaCT has no role in the entry of acetate into hepatocytes. Extracellular acetate was incorporated into lipids extractable with *n*-hexane/propan-2-ol as expected, but Li⁺ did not have any effect on this process. These data show that Li⁺ at therapeutic concentrations enhances the utilization of extracellular citrate in human liver cells for the synthesis of fatty acids and cholesterol specifically via stimulation of NaCT.

To determine the domains in human NaCT that are responsible for the stimulation of transport function by Li⁺, we made 28 different chimaeric transporters consisting of different regions of human and rat NaCTs, and compared their transport function with that of the parent rat and human NaCTs (results not shown). The results of these studies have shown that replacement of a small region (amino acid positions 496–516) in human NaCT with the corresponding region (amino acid positions 500–520) in rat NaCT stimulated the transport function 5-fold, and that the activity of this chimaeric transporter was not affected by Li⁺ (Figure 4A). There are only two amino acid differences between human and rat NaCTs in this region (Phe⁵⁰⁰ versus Leu⁵⁰⁴, and Thr⁵¹⁶ versus Ala⁵²⁰) (Figure 4B). We mutated codon-500 and codon-516 individually in human NaCT to generate the Phe → Leu and Thr → Ala mutants that match the sequence in rat NaCT of this region. The Thr → Ala mutation did not affect the transport activity; nor did it interfere with the Li⁺-dependent stimulation (results not shown). In contrast,

Figure 4 Structure–function relationship for NaCT

(A) Influence of Li⁺ (10 mM) on the uptake of citrate (20 μ M) via wild-type human and rat NaCTs, and the chimaeric transporter in which the region containing the amino acids 496–516 in human NaCT has been replaced with the corresponding region from rat NaCT (shown by the grey-shaded bar). (B) Comparison of the amino acid sequences between human NaCT (amino acids 496–516) and rat NaCT (amino acids 500–520). The amino acids that are different between human and rat NaCTs are identified in bold typeface. (C) Substrate saturation kinetics of wild-type human NaCT (●) and the Phe → Leu mutant of human NaCT (○). The inset shows an Eadie–Hofstee plot of the same data. (D) Influence of increasing concentrations of Li⁺ on the uptake of citrate (20 μ M) via wild-type human NaCT (●) and the Phe → Leu mutant of human NaCT (○).

the Phe → Leu mutation (TTT → CTT) led to a 3- to 4-fold stimulation of transport activity. Kinetic analysis of the wild-type human NaCT and its Phe → Leu mutant showed that the stimulation of transport function seen with the mutant was due to an increase in substrate affinity, with very little change in maximal velocity (Figure 2C). The wild-type human NaCT showed a K_t value of $585 \pm 87 \mu$ M for citrate. The corresponding value for the mutant was $78 \pm 8 \mu$ M. Thus the Phe → Leu mutant showed a 7-fold increase in substrate affinity. Since a single base-pair change in the codon can bring about this amino acid substitution (TTT → CTT), these findings suggest that genetic mutations in humans have potential to effect marked changes in the constitutive activity of NaCT, and that such changes may influence the role of the transporter in hepatic synthesis of triacylglycerols and cholesterol. Interestingly, the Phe → Leu mutant did not exhibit Li⁺-sensitivity (Figure 2D). The transport function of wild-type

human NaCT was stimulated by Li^+ , whereas the transport function of the mutant, which was about 4-fold higher than that of wild-type NaCT, was not affected by Li^+ . Since rat NaCT, which possesses leucine at this site, is inhibited by Li^+ , the finding that the activity of the Phe $\rightarrow$ Leu mutant of human NaCT is not inhibited by Li^+ indicates that this region may not represent the Li^+ -binding site. Furthermore, Li^+ affects the transport function of human NaCT, not only by increasing the substrate affinity, but also by decreasing the maximal velocity (Figure 2A). In contrast, the influence of the Phe $\rightarrow$ Leu mutation is solely on substrate affinity. We predict that, although the domain in human NaCT consisting of the amino acids 496–516 definitely has a critical role in substrate binding, it may not have a direct role in Li^+ binding. However, this domain appears to interact with the Li^+ -binding site, thus modifying the influence of Li^+ on the transport function. Multiple alignment of amino acid sequences of NaCTs from different species using the program 'T-coffee' (<http://www.ch.embnet.org/software/TCoffee.html>) shows that the amino acid corresponding to Phe⁵⁰⁰ in human NaCT is a leucine residue in rat and mouse NaCTs. Interestingly, the corresponding amino acid in zebrafish and *C. elegans* NaCTs is phenylalanine. Nevertheless, the zebrafish NaCT is inhibited by Li^+ , whereas that from *C. elegans* is not influenced by Li^+ . This agrees with our conclusion that the domain containing this amino acid does not represent the Li^+ -binding site.

Our studies showing that Li^+ is a potent activator of NaCT at concentrations found in patients treated with lithium for affective disorders have important clinical implications. Interestingly, the activation of NaCT by Li^+ is seen only with human NaCT. Patients with bipolar disorder are treated effectively with either lithium or other mood stabilizers, such as valproate and carbamazepine. The present study has shown that the activation of human NaCT is seen only with lithium, and not with valproate and carbamazepine. This means that only those patients who take lithium would face the clinical consequences of activation of NaCT function. We hypothesize that patients with affective disorders who are taking lithium may have enhanced synthesis of cholesterol and fatty acids, and consequently exhibit increased risk for hypercholesterolaemia, hyperlipidaemia, hyperglycaemia, obesity and insulin-resistant diabetes.

Inositol monophosphatase, inositol polyphosphatase and glycogen synthase kinase-3 β , the currently known targets for Li^+ , are all enzymes and are inhibited by Li^+ . Human NaCT is, in contrast, a plasma membrane transporter that is activated, rather than inhibited, by Li^+ . Human NaCT thus represents the newest, and a novel, target for Li^+ . NaCT is expressed in the brain, where it is localized in neurons [4]. Therefore, at therapeutic concentrations, Li^+ can be expected to facilitate the cellular entry of citrate and improve the energy status of the neurons. In addition, brain possesses significant capacity to synthesize cholesterol [19,20], and NaCT in neurons may mediate the utilization of extracellular citrate for the synthesis of cholesterol. Thus Li^+ may facilitate the local synthesis of cholesterol in the brain. However, in spite of these potential physiological functions of NaCT in the brain, it is unlikely that the activation of NaCT by Li^+ is solely responsible for the therapeutic actions of Li^+ . Valproate and carbamazepine are equally effective in the treatment of bipolar disorder, but these drugs do not have any noticeable effect on NaCT. On the other hand, the activation of NaCT by Li^+ in the liver may have significant clinical consequences. There has been some interest in the past in the modulation of carbohydrate metabolism by Li^+ , but the data are conflicting. Some studies have shown that lithium therapy in humans enhances glucose disposal and improves glucose tolerance [21], whereas other studies have reported the opposite result, in which lithium

therapy led to hyperglycaemia [22]. There is no information on the influence of lithium therapy on blood levels of fatty acids and cholesterol. There is strong evidence, however, that lithium therapy is associated with a significant increase in body weight [23–28]. Whether the facilitation of fatty acid synthesis in human liver by Li^+ via NaCT has any relevance to this phenomenon remains to be determined.

The results with the Phe $\rightarrow$ Leu mutant of human NaCT raise the possibility that genetic mutations in this amino acid position may alter the constitutive activity of the transporter in humans, and consequently influence the hepatic utilization of extracellular citrate for fatty acid and cholesterol synthesis. Individuals with such mutations are likely to exhibit significant alterations in their blood-lipid profile.

REFERENCES

- Inoue, K., Fei, Y. J., Huang, W., Zhuang, L., Chen, Z. and Ganapathy, V. (2002) Functional identity of *Drosophila melanogaster* Indy as a cation-independent, electroneutral transporter for tricarboxylic acid-cycle intermediates. *Biochem. J.* **367**, 313–319
- Knauf, F., Rogina, B., Jiang, Z., Aronson, P. S. and Helfand, S. L. (2002) Functional characterization and immunolocalization of the transporter encoded by the life-extending gene *indy*. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 14315–14319
- Rogina, B., Reenan, R. A., Nilsen, S. P. and Helfand, S. L. (2000) Extended life span conferred by co-transporter gene mutations in *Drosophila*. *Science* **290**, 2137–2140
- Inoue, K., Zhuang, L., Maddox, D. M., Smith, S. B. and Ganapathy, V. (2002) Structure, function, and expression pattern of a novel sodium-coupled citrate transporter (NaCT) cloned from mammalian brain. *J. Biol. Chem.* **277**, 39469–39476
- Inoue, K., Zhuang, L. and Ganapathy, V. (2002) Human Na⁺-coupled citrate transporter: structure, genomic organization and transport function. *Biochem. Biophys. Res. Commun.* **299**, 465–471
- Pajor, A. M. (2000) Molecular properties of sodium/dicarboxylate cotransporters. *J. Membr. Biol.* **175**, 1–8
- Wang, H., Fei, Y. J., Kekuda, R., Yang-Feng, T. L., Devoe, L. D., Leibach, F. H., Prasad, P. D. and Ganapathy, V. (2000) Structure, function, and genomic organization of human Na⁺-dependent dicarboxylate transporter. *Am. J. Physiol.* **278**, C1019–C1030
- Bond, P. A., Jenner, F. A., Lee, C. R., Lenton, E., Pollitt, R. J. and Sampson, G. A. (1972) The effect of lithium salts on the urinary excretion of α -ketoglutarate in man. *Br. J. Pharmacol.* **46**, 116–123
- Wright, E. M., Wright, S. H., Hirayama, B. and Kippen, I. (1982) Interactions between lithium and renal transport of Krebs cycle intermediates. *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7514–7517
- Bode, B. P., Fuchs, B. C., Hurley, B. P., Conroy, J. L., Suetterlin, J. E., Tanabe, K. K., Rhoads, D. B., Abcouwer, S. F. and Soubra, W. W. (2002) Molecular and functional analysis of glutamine uptake in human hepatoma and liver-derived cells. *Am. J. Physiol.* **283**, G1062–G1073
- Schramagl, H., Schinker, R., Gierens, H., Nauck, M., Weiland, H. and Marz, W. (2001) Effect of atorvastatin, simvastatin, and lovastatin on the metabolism of cholesterol and triacylglycerides in HepG2 cells. *Biochem. Pharmacol.* **62**, 1545–1555
- Sproule, B. (2002) Lithium in bipolar disorder: can drug concentrations predict therapeutic effect? *Clin. Pharmacokinet.* **41**, 639–660
- Nordmann, J. and Nordmann, R. (1961) Organic acids in blood and urine. *Adv. Clin. Chem.* **4**, 53–120
- Williams, R. S., Cheng, L., Mudge, A. W. and Harwood, A. J. (2002) A common mechanism of action for three mood-stabilizing drugs. *Nature (London)* **417**, 292–295
- Jungermann, K. and Katz, N. (1989) Functional specialization of different hepatocyte populations. *Physiol. Rev.* **69**, 708–764
- Williams, R. S. and Harwood, A. J. (2000) Lithium therapy and signal transduction. *Trends Pharmacol. Sci.* **21**, 61–64
- Phiel, C. J. and Klein, P. S. (2001) Molecular targets of lithium action. *Annu. Rev. Pharmacol. Toxicol.* **41**, 789–813
- Li, X., Bijur, G. N. and Jope, R. S. (2002) Glycogen synthase kinase-3 β , mood stabilizers, and neuroprotection. *Bipolar Disord.* **4**, 137–144
- Spady, D. K. and Dietschy, J. M. (1983) Sterol synthesis *in vivo* in 18 tissues of the squirrel monkey, guinea pig, rabbit, hamster, and rat. *J. Lipid Res.* **24**, 303–315
- Dietschy, J. M., Turley, S. D. and Spady, D. K. (1993) Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *J. Lipid Res.* **34**, 1637–1659
- Heninger, G. R. and Mueller, P. S. (1970) Carbohydrate metabolism in mania. *Arch. Gen. Psychiatry* **23**, 310–318

-
- 22 Shopsin, B., Stern, S. and Gershon, S. (1972) Altered carbohydrate metabolism during treatment with lithium carbonate. *Arch. Gen. Psychiatry* **26**, 566–570
- 23 Chen, Y. and Silverstone, T. (1990) Lithium and weight gain. *Int. Clin. Psychopharmacol.* **5**, 217–225
- 24 Coxhead, N., Silverstone, T. and Cookson, J. (1992) Carbamazepine versus lithium in the prophylaxis of bipolar affective disorder. *Acta Psychiatr. Scand.* **85**, 114–118
- 25 Price, L. H. and Heninger, G. R. (1994) Lithium in the treatment of mood disorders. *N. Engl. J. Med.* **331**, 591–598
- 26 Baptista, T., Teneud, L., Contreras, Q., Alastre, T., Burguera, J. L., de Burguera, M., de Baptista, E., Weiss, S. and Hernandez, L. (1995) Lithium and body weight gain. *Pharmacopsychiatry* **28**, 35–44
- 27 Fagiolini, A., Frank, E., Houck, P. R., Mallinger, A. G., Swartz, H. A., Buysse, D. J., Ombao, H. and Kupfer, D. J. (2002) Prevalence of obesity and weight change during treatment in patients with bipolar disorder. *J. Clin. Psychiatry* **63**, 528–533
- 28 Atmaca, M., Kuloglu, M., Tezcan, E. and Ustundag, B. (2002) Weight gain and serum leptin levels in patients on lithium treatment. *Neuropsychobiology* **46**, 67–69

Received 4 June 2003/18 June 2003; accepted 24 June 2003

Published as BJ Immediate Publication 26 June 2003, DOI 10.1042/BJ20030827