

ESTROGEN REGULATION AND ION DEPENDENCE OF TAURINE UPTAKE BY MCF-7 HUMAN BREAST CANCER CELLS

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Abstract: It has been reported that estrogen receptor-positive MCF-7 cells express TauT, a Na⁺-dependent taurine transporter. However, there is a paucity of information relating to the characteristics of taurine transport in this human breast cancer cell line. Therefore, we have examined the characteristics and regulation of taurine uptake by MCF-7 cells. Taurine uptake by MCF-7 cells showed an absolute dependence upon extracellular Na⁺. Although taurine uptake was reduced in Cl⁻ free medium a significant portion of taurine uptake persisted in the presence of NO₃⁻. Taurine uptake by MCF-7 cells was inhibited by extracellular β-alanine but not by L-alanine or L-leucine. 17β-estradiol increased taurine uptake by MCF-7 cells: the V_{max} of influx was increased without affecting the K_m. The effect of 17β-estradiol on taurine uptake by MCF-7 cells was dependent upon the presence of extracellular Na⁺. In contrast, 17β-estradiol had no significant effect on the kinetic parameters of taurine uptake by estrogen receptor- negative MDA-MB-231 cells. It appears that estrogen regulates taurine uptake by MCF-7 cells via TauT. In addition, Na⁺-dependent taurine uptake may not be strictly dependent upon extracellular Cl⁻.

Key words: Breast cancer, Taurine uptake, Estrogen

INTRODUCTION

Taurine (2-aminoethanesulfonic acid), a sulfur-containing β-amino acid, is one of the most abundant free amino acid within the cell interior [1]. Many functions have been ascribed to taurine including a role in cell volume regulation, bile salt

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Abbreviations used: MOPS – 3-(N-Morpholino)-propanesulfonic acid; TauT – taurine transporter; Tris – Tris[hydroxymethyl]aminoethane

conjugation, antioxidation, modulation of calcium channels, detoxification and immunomodulation [1]. The requirements for taurine are met from dietary sources and/or from biosynthesis using cysteine and methionine as precursors. Some species, including humans have a limited capacity to synthesize taurine whereas others, such as the rat, can readily synthesize significant quantities [1].

The mechanism of taurine transport into cells has received considerable attention because of its important role in the regulation of cell function [2]. High-affinity taurine uptake is mediated by a mechanism originally designated as System β and whose molecular correlate is TauT [4-6]. TauT is a protein that has a molecular weight of 65-74 kDa and is predicted to have 12 membrane-spanning domains. Saturable taurine uptake by mammalian cells displays an absolute dependency upon extracellular Na^+ : replacing Na^+ with choline almost abolishes carrier-mediated taurine uptake. The role of Cl^- in taurine transport is at present unclear. On the one hand there is evidence to suggest that TauT may be a (Na^+ - Cl^- - taurine) cotransporter [4, 6]. On the other hand, Uchida et al [3] and Vinnakota et al [7] have respectively shown that SCN^- and formate can support a significant taurine influx via TauT. Other studies have demonstrated that a substantial portion of cellular taurine uptake, presumably via TauT, persists when extracellular NO_3^- replaces Cl^- [8-11].

TauT is widely expressed in epithelial tissues including the human breast [5]. In this connection it has been reported that an estrogen receptor-positive human breast cancer cell line, namely MCF-7, expresses TauT [12]. It is apparent that TauT is transactivated by 17β -estradiol and p53 in MCF-7 cells. It has been reported that rodent mammary tumors have high levels of intracellular taurine [13]. Furthermore, Beckonert et al [14] have shown that the intracellular concentration of taurine, measured by NMR spectroscopy, is increased in breast tumours compared with that found in healthy breast tissue. Indeed, it has been suggested that the intracellular concentration of taurine may be a potential indicator of tumour aggressiveness [15].

Despite these important findings there appears to be a paucity of information relating to the functional properties of taurine transport and its regulation in breast cancer cells. In this study we have examined the ion dependence of taurine transport and its regulation by 17β -estradiol in MCF-7 cells. The results show that the Na^+ -dependent uptake of taurine by MCF-7 cells is stimulated by 17β -estradiol. The results also show that Na^+ -dependent taurine uptake into MCF-7 cells, presumably via TauT, is not entirely Cl^- -dependent.

MATERIALS AND METHODS

Materials

1,2- ^3H]taurine (specific activity 29 Ci/mmol) was purchased from Amersham plc, Bucks, UK. All other chemicals were obtained from Sigma, Poole, Dorset, UK.

Cell culture

MCF-7 and MDA-MB-231 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with L-glutamine (2 mM), heat-inactivated fetal bovine serum (10%), penicillin (50 IU/ml) and streptomycin (50 µg/ml). Cells were seeded in 35-mm culture wells containing 2 ml of culture DMEM medium (with supplements) at a density of $0.3-1.0 \times 10^6$ cells per well. The cells were cultured for 1-3 days at 37°C in a gas phase of air with 5% CO₂ and were used at 60-90% confluency. The effect of 17β-estradiol was examined in cells cultured in serum and phenol red-free DMEM (but including the other supplements). 17β-Estradiol was dissolved in ethanol to give a stock solution of 10 µM. A 1:1000 dilution of the stock solution was then made to give a final concentration of 10 nM in 2 ml of the cell culture medium. A similar amount of ethanol was added to the controls.

Taurine uptake

The uptake of taurine by MCF-7 and MDA-MB-231 cells was assayed using [³H]taurine as tracer. The culture medium was removed and the cells were washed three times with 3 ml of a buffer containing (mM) 135 NaCl, 5 KCl, 2 CaCl₂, 1 MgSO₄, 10 glucose and 10 Tris-MOPS, pH 7.4. Following, this, the cells were pre-incubated for 5 min at 37°C in 2ml of an appropriate buffer (see figure legends for details of precise composition). The measurement of radiolabelled taurine uptake was commenced by removing the pre-incubation buffer and replacing it with 1 ml of a buffer similar in composition except that it also contained 1µCi/ml of [³H]taurine. At predetermined times, the incubation buffer was removed and the cells were washed four times with 3 ml of an ice-cold buffer containing (mM) 135 NaCl, 5 KCl, 2 CaCl₂, 1 MgSO₄, 10 glucose and 10 Tris-MOPS, pH 7.4. The cells were lysed by incubating in 1 ml of 50 mM NaOH for at least 3 hours. A portion of the lysate (0.5 ml) was added to 10 ml of UltimaGold liquid scintillation cocktail. Samples of each lysate (100 µl) were taken to determine the protein content using the Lowry assay. The specific activity of [³H]taurine in the incubation medium was determined by counting the radioactivity associated with 10 µl of buffer. The uptake of taurine by the cells was expressed as pmoles/mg protein. Preliminary experiments revealed that the uptake of taurine into both MCF-7 and MDA-MB-231 cells was linear for at least 15 min (results not shown). Therefore, in subsequent experiments taurine uptake was measured after 10 min of incubation. The osmolality of the buffers were routinely checked using an MicroOsmometer (Vitech Scientific LTD, UK).

Data analysis

Statistical differences were assessed using Student's paired or unpaired t-test as appropriate and were considered significant when $P < 0.05$. Kinetic parameters of taurine uptake (ie V_{max} and K_m) were obtained by fitting the data to the Michaelis-Menten equation using non-linear regression analysis.

RESULTS

Ion dependence of taurine uptake

The first step in the investigation was to examine the effect of replacing Na^+ and Cl^- on taurine uptake by MCF-7 cells. Fig. 1A illustrates that substituting choline for Na^+ markedly reduced taurine influx ($P < 0.01$): choline only supported 3.3%

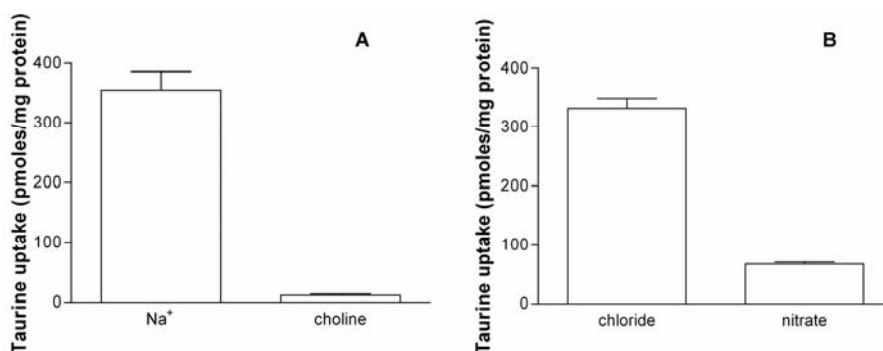


Fig. 1. The influence of Na^+ and Cl^- on taurine uptake by MCF-7 cells. A – The effect of replacing extracellular Na^+ on taurine uptake by MCF-7 cells. The incubation medium contained (mM) 0.01 taurine, 135 NaCl or choline Cl, 5 KCl, 2 CaCl_2 , 1 MgSO_4 , 10 glucose and 10 Tris-MOPS pH 7.4. Uptake was measured at 37°C after 10 minutes of incubation. Values are the means \pm SE of 3 experiments. B – The effect of replacing extracellular Cl^- on taurine uptake by MCF-7 cells. The incubation medium contained (mM) 0.01 taurine, 135 NaCl or NaNO_3 , 5 KCl or KNO_3 , 2 CaCl_2 or $\text{Ca}(\text{NO}_3)_2$, 1 MgSO_4 , 10 glucose and 10 Tris-MOPS, pH 7.4. Uptake was measured at 37°C after 10 min of incubation. Values are the means \pm SE of 3 experiments.

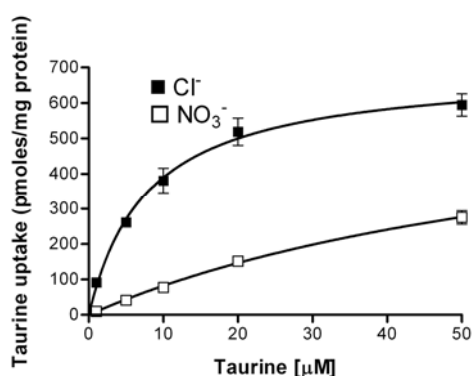


Fig. 2. The kinetics of taurine uptake by MCF-7 cells in the presence of extracellular Cl^- or NO_3^- . The incubation medium contained (mM) 0.001 – 0.05 taurine, 135 NaCl of NaNO_3 , 5 KCl or KNO_3 , 2 CaCl_2 or $\text{Ca}(\text{NO}_3)_2$, 1 MgSO_4 , 10 glucose and 10 Tris-MOPS, pH 7.4. Uptake was measured at 37°C after 10 min of incubation. Values are the means \pm SE of 3 experiments.

of the total flux. Fig. 1B shows that replacing extracellular Cl^- with NO_3^- reduced taurine uptake ($P < 0.01$) by MCF-7 cells but not to the same extent as replacing extracellular Na^+ (Fig. 1A). Thus, NO_3^- was able to support 20% of the flux found in the presence of Cl^- .

We examined the kinetic parameters of taurine uptake by MCF-7 cells with respect to the extracellular concentration of taurine in the presence and absence of extracellular Cl^- (Fig. 2). In this set of experiments the extracellular concentration of taurine ranged from 1 to 50 μM . In the presence of Cl^- , taurine uptake could be described by a single-saturable curve having an apparent K_m of $8.1 \pm 1.0 \mu\text{M}$ and V_{\max} 702.1 ± 45.6 pmoles/mg protein/10 min (\pm S.E., $n = 3$). Replacing extracellular Cl^- with NO_3^- lowered the affinity ($P < 0.001$) of the carrier for taurine without affecting the V_{\max} of uptake. Thus, the apparent K_m and V_{\max} in the presence of NO_3^- were respectively $72.1 \pm 1.8 \mu\text{M}$ and 675.1 ± 57.9 pmoles/mg protein/10 min (\pm S.E., $n = 3$).

Substrate specificity of taurine uptake

We examined the substrate specificity of the taurine transporter in MCF-7 cells by testing the effect of other amino acids, added to the incubation medium at a concentration of 1 mM, on taurine influx. The results shown in Fig. 3 suggest that the taurine carrier in MCF-7 cells is specific for β -amino acids. Thus, β -alanine added to the incubation medium markedly reduced taurine uptake by $90.4 \pm 1.9\%$ (\pm S.E., $n = 3$, $P < 0.05$) whereas L-alanine and L-leucine had no significant effect.

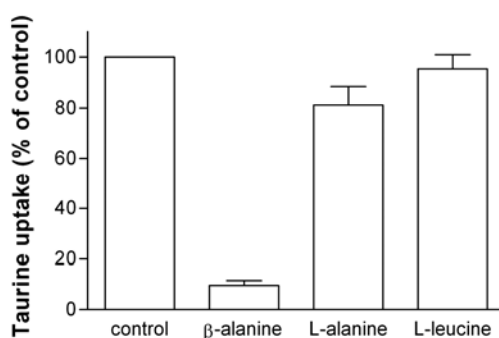


Fig. 3. The effect of β -alanine, L-alanine and L-leucine on taurine uptake by MCF-7 cells. The incubation medium contained (mM) 0.01 taurine, 135 NaCl, 5 KCl, 2 CaCl_2 , 1 MgSO_4 , 10 glucose and 10 Tris-MOPS pH 7.4. When required, β -alanine, L-alanine or L-leucine were added to the medium to give a final concentration of 1 mM. Uptake was measured at 37°C after 10 min of incubation. Values are the means \pm SE of 3 experiments.

The effect of 17 β -estradiol on taurine uptake

Taurine uptake by MCF-7 cells incubated in the absence or presence of 17 β -estradiol (10 nM) was examined. In this set of experiments the cells were cultured in phenol red- and serum-free medium for 24 h prior to the addition of the hormone (or ethanol vehicle). Following this, the cells were cultured in the absence or presence of 10 nM 17 β -estradiol for a further 48 h prior to assaying

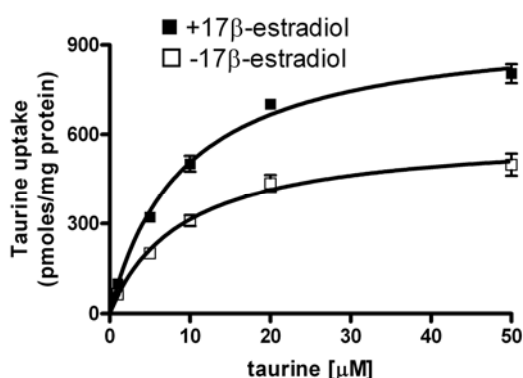


Fig. 4. Taurine uptake by MCF-7 cells which had been incubated in the presence or absence of 17 β -estradiol (10nM). The incubation medium contained (mM) 0.001-0.05 taurine, 135 NaCl, 5 KCl, 2 CaCl₂, 1MgSO₄, 10 glucose and 10 Tris-MOPS, pH 7.4. Uptake was measured at 37°C after 10 min of incubation. Values are means \pm SE of 5 experiments.

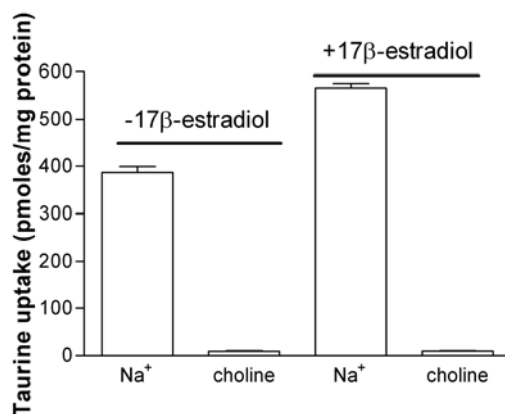


Fig. 5. The effect of replacing extracellular Na⁺ on taurine uptake by MCF-7 cells which had been incubated in the presence or absence of 17 β -estradiol (10nM). The incubation medium contained (mM) 0.01taurine, 135 NaCl or Choline-Cl, 5 KCl, 2 CaCl₂, 1 MgSO₄, 10 glucose and 10 Tris-MOPS, pH 7.5. Uptake was measured at 37°C after 10 min of incubation. Values are means \pm SE of 3 experiments.

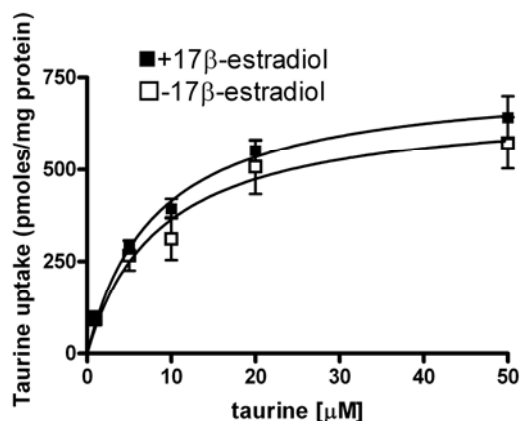


Fig. 6 Taurine uptake by MDA-MB-231 cells which had been incubated in the presence or absence of 17 β -estradiol (10nM). The incubation medium contained (mM) 0.001-0.05 taurine, 135 NaCl, 5 KCl, 2 CaCl₂, 1 MgSO₄, 10 glucose and 10 Tris-MOPS, pH 7.4. Values are the means \pm SE of 4 experiments.

taurine uptake. The results of these experiments are shown in Fig. 4. 17 β -Estradiol increased the V_{\max} of taurine uptake ($P < 0.001$). Thus, the V_{\max} of taurine influx in the presence and absence of 17 β -estradiol was respectively 983.9 ± 58.4 and 607.5 ± 53.9 pmoles/mg protein/10 min (\pm SE, $n = 5$). In contrast, 17 β -estradiol had no significant effect on the K_m of taurine influx (9.6 ± 1.4 v. 9.3 ± 1.1 μM). Fig. 5 reveals that 17 β -estradiol increased the Na⁺-dependent component of taurine uptake by MCF-7 cells ($P < 0.001$) whilst having no effect on the Na⁺-independent influx of taurine. On the other hand, 17 β -estradiol had no significant effect on the kinetic parameters of taurine uptake by the estrogen receptor-negative MDA-MB-231 human breast cancer cell line (Fig. 6). The V_{\max} of taurine uptake by MDA-MB-231 cells in the presence and absence of 17 β -estradiol was respectively 757.6 ± 58.9 and 687.4 ± 78.5 pmoles/mg protein/10 min (\pm SE, $n = 4$). The K_m of taurine uptake by MDA-MB-231 cells was also unaffected by 17 β -estradiol: the K_m in the presence and absence of the hormone was respectively 8.4 ± 1.2 and 9.4 ± 1.3 μM (\pm SE, $n = 4$).

DISCUSSION

The effect of estrogen on taurine uptake

The results show for the first time that taurine uptake by MCF-7 is via a Na⁺-dependent mechanism which is up-regulated by estrogen. There are several lines of evidence suggesting that taurine uptake by MCF-7 cells is via TauT. Firstly, several of the characteristics of taurine uptake by MCF-7 cells described in this study reflect those of the cloned transporter. Thus, taurine uptake shows an absolute dependency upon extracellular Na⁺ and appears to be selective for

β -amino acids. Furthermore the transporter operates with high-affinity with respect to extracellular taurine which suggests that the transporter is TauT rather than any of the cloned GABA transporters [16]. Secondly, we found that 17β -estradiol increased the V_{\max} of taurine uptake which is in accordance with the earlier observations by Han et al [12] that estrogen increases the expression of TauT in MCF-7 cells. Indeed, the finding that the estrogen-sensitive portion of taurine uptake by MCF-7 cells was Na^+ -dependent suggests that 17β -estradiol is acting to increase taurine uptake via TauT. Importantly, the effect of estrogen on taurine uptake by MCF-7 cells appears to be specific since the hormone had no effect on the kinetic parameters of taurine uptake by estrogen receptor-negative MDA-MB-231 cells. This suggests that estrogen is acting via its cognate receptor to increase taurine transport in MCF-7 cells.

Estrogen has been shown to stimulate amino acid uptake via other transporters in MCF-7 cells. For example, the expression and activity of system A, a Na^+ -dependent transporter of small neutral amino acids, is increased by estrogen. Bhat & Vadgama [12] found that the uptake (V_{\max}) of methylaminoisobutyric acid (MeAIB), a paradigm substrate of system A, was almost doubled by incubating MCF-7 cells in the presence of 10nM 17β -estradiol. Furthermore, L-leucine transport via system L (most likely the LAT1 isoform) in MCF-7 cells is increased by estrogen: the V_{\max} of BCH-inhibitable L-leucine uptake was increased by 50% by 10 nM 17β -estradiol [18]. However, the effect of estrogen on amino acid transporters in MCF-7 cells appears to be relatively specific: estrogen had no effect on arginine and threonine uptake via systems y^+ and ASC respectively [17].

There is now evidence for an effect of estrogen on at least three specific and diverse amino acid transport systems in MCF-7 cells. Therefore, it is tempting to suggest that estrogen contributes to the proliferative capacity of estrogen receptor-positive breast cancer cells by increasing amino acid uptake. This adds further weight to the suggestion that amino acid transport mechanisms are an attractive target to limit the growth of breast cancer cells [18]. Amino acid transporters, especially those that have essential amino acids as substrates, may be effective therapeutic targets [19].

Intracellular amino acids, particularly taurine, play an important role in regulating the cell volume which in turn acts as a signal to control several important metabolic processes such as protein and lipid synthesis [20-22]. Taurine uptake via TauT could act to maintain the intracellular levels of taurine which subsequently could be used to help MCF-7 cells regulate their volume. In this connection we have evidence that an increase in the cellular hydration state of MCF-7 cells leads to a rapid increase in taurine release from the intracellular compartment via a pathway which is inhibited by DIDS [23]. Therefore, inhibition of taurine uptake by breast cancer cells could compromise cell volume regulation and thus cell survival given that the cellular hydration state is linked to a number of vital processes.

The Cl⁻-dependence of taurine uptake

It is beyond doubt that high-affinity concentrative taurine uptake by mammalian cells has an absolute requirement for extracellular Na⁺. Accordingly, TauT displays a strong dependency on Na ions [3, 4, 6]. Indeed, in the absence of extracellular Na⁺ there is negligible taurine uptake. There is also a general consensus that the β -amino acid transporter requires Cl⁻ for optimal activity and therefore could be (Na⁺-Cl⁻- taurine) cotransport system. However, the results of the present study suggest that the Na⁺-dependent taurine transporter may not have a strict dependence on Cl⁻ as previously assumed. We found that NO₃⁻ was able to support a considerable flux of taurine into MCF-7 cells albeit with reduced affinity. Nitrate was able to support 46% of uptake when taurine was present in the incubation medium at the near-physiological concentration of 50 μ M.

A close inspection of the literature reveals that there are several instances whereby NO₃⁻ is able to partially substitute for Cl⁻ in supporting Na⁺-dependent taurine influx. For example, more than 30% of Na⁺-dependent taurine uptake by skate hepatocytes persists in a Cl⁻- free (NO₃⁻- replacement) buffer [9]. Similarly, a large portion of the Na⁺-dependent uptake of taurine by Ehrlich Ascites tumour cells (25%) and human glioma GL15 cells (29%) remains in a NO₃⁻ buffer [8, 24]. In addition, it has been reported that SCN⁻ is able to support a substantial portion of taurine uptake via TauT [3]. On the other hand, there are several reports showing that replacing Cl⁻ with gluconate leads to a fall in taurine uptake similar to that seen under Na⁺-free conditions [4, 6]. Gluconate, unlike NO₃⁻ and SCN⁻, is an impermeable anion, therefore, it may simply be that the Na⁺-dependent taurine uptake via TauT requires the presence of a permeable anion and is not an obligatory (Na⁺-Cl⁻-taurine) cotransporter.

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