Osmoregulation of Taurine Efflux from Cultured Human Breast Cancer Cells: Comparison with Volume Activated Cl⁻ Efflux and Regulation by Extracellular ATP

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Key Words

Taurine efflux • Breast cancer cells • Volume-regulation

Abstract

The properties and regulation of volume-activated taurine efflux from MDA-MB-231 and MCF-7 cells have been investigated. Volume-activated taurine release from both cell lines was almost completely inhibited by diidosalicylate. DIDS, was more effective at inhibiting swelling-induced taurine release from MCF-7 than from MDA-MB-231 cells. On the basis of comparing taurine, Cl⁻ and I⁻ efflux time courses, it appears that volume-activated taurine efflux does not utilize volume-sensitive anion channels in MDA-MB-231 and MCF-7 cells. Extracellular ATP stimulated volume-activated taurine release from MDA-MB-231 cells but not from MCF-7 cells. The effect of ATP was mimicked by UTP and was dependent upon external calcium and inhibited by suramin. However, suramin inhibited volume-activated taurine efflux from both MDA-MB-231 and MCF-7 cells even in the absence of exogenously added ATP suggesting that it acts directly on the taurine efflux pathway and/or is inhibiting the effect of ATP released from the cells. Volume-

activated taurine efflux from MDA-MB-231 cells was stimulated by ionomycin. In contrast, ionomycin had no effect on taurine release from MCF-7 cells. Adenosine also stimulated volume-activated taurine efflux from MDA-MB-231 cells. The results suggest that purines regulate taurine transport in MDA-MB-231 cells via more than one type of receptor.

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Introduction

Cell volume is constantly challenged by factors which change either the intra- or extracellular osmolality. Thus, cell volume can be altered by exposing cells to anisotonic conditions or by changing the rate of substrate transport and oxidative metabolism [1]. Cells are, however, able to regulate their volume following a change to their hydration state. This is accomplished by a net efflux or uptake of solutes together with osmotically obliged water [2]. For example, membrane transport processes involved in volume regulation following cell swelling include K⁺ and Cl⁻ channels, (K⁺-Cl⁻) co-transport and a mechanism which transports organic osmolytes, including amino acids.

The β -amino acid taurine is a paradigm substrate of the volume-activated organic osmolyte efflux pathway [3, 4]. The nature of the volume-activated taurine efflux pathway is, however, a matter of controversy. On the one hand it has been suggested that swelling-induced taurine release utilizes volume-activated chloride channels (eg see [5, 6]). On the other hand there is evidence suggesting that volume-activated taurine efflux is independent from chloride channels (eg see [7-9]).

Although cells have to regulate their volume within relatively narrow limits to survive, it is apparent that cell volume *per se* is an important signalling factor involved in the regulation of key metabolic processes such as protein synthesis and lipogenesis [10]. The activity and regulation of volume-activated membrane transport systems play a key role in the control of cell growth and proliferation. It is also becoming apparent that cell volume, and thus volume-activated solute transport, plays a paramount role in programmed cell death [11]. It appears that a reduction of cell volume under isosmotic conditions, induced by the activation of transport mechanisms normally activated by cell swelling, is one of the earliest events in the apoptotic cascade [11, 12].

A thorough understanding of the mechanisms underlying volume regulation could help develop strategies aimed at controlling the growth of tumour cells. In this regard, we are investigating the properties and regulation of volume-activated transport processes in two cultured human breast cancer cell lines, namely, MDA-MB-231 and MCF-7 cells which are established models for estrogen receptor-negative and -positive breast cancer cells respectively. We have recently studied the regulation of K^+ transport in these cells [13]. In this report we describe swelling-induced taurine efflux from MDA-MB-231 and MCF-7 cells. In particular we have investigated the relationship between taurine efflux and chloride channels and the regulation of volume-activated taurine release by extracellular ATP.

Materials and Methods

Cell Culture

MDA-MB-231 and MCF-7 cells were cultured in Dulbeccos Modified Eagle Medium (DMEM) supplemented with L-glutamine (2 mM), heat-inactivated fetal bovine serum (10%), penicillin (50 IU/ml) and streptomycin (50 μ g/ml). Cells were cultured in a gas phase of air with 5% CO_2 at 37°C. Cells were seeded in 35 mm culture wells containing 2 ml of culture medium and were used when they had reached 60-90% confluency.

Taurine efflux

Taurine efflux was measured from MDA-MB-231 and MCF-7 cells using a modification of the method described by Shennan et al [14]. Briefly, cells were loaded with radiolabelled taurine by incubating in a buffer containing (mM) 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 10 glucose and 10 Tris-MOPS, pH 7.4 plus 1 μ Ci/ml [3H]taurine (35 nM) for 60 min at 37°C. The loading buffer was aspirated and the cells were washed four times in rapid succession with a buffer similar in composition to that just described except that it contained no radioactivity. The efflux of radiolabelled taurine from the cells was measured by the sequential addition and removal of 2 ml of medium (see figure legends for precise details of composition) at 1 min intervals at 37°C. The cells were lysed after the wash-out period by incubating in 2 ml of distilled water for at least 3 h. The wash-out samples and the lysate were prepared for counting by adding 10 ml of UltimaGold liquid scintillation cocktail. The fractional efflux for each collection period was calculated according to the method described by Shennan et al [8]. Preliminary experiments established that taurine efflux from MDA-MB-231 and MCF-7 cells, measured under isosmotic conditions, followed first order kinetics and thus could be described by a single mono-exponential equation (results not shown). In subsequent experiments, taurine efflux was allowed to proceed for 4 min prior to testing the effect of a hyposmotic shock.

Chloride and Iodide efflux

Chloride and iodide efflux was measured from MDA-MB-231 and MCF-7 cells respectively using ^{36}Cl and ^{125}I as tracers. The cells were loaded with the isotopes by incubating in a buffer for 60 min (^{125}I) or 120 min (^{36}Cl) containing (mM) 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 10 glucose and 10 tris MOPS, pH 7.4 plus 3.0 μ Ci/ml ^{36}Cl or 2.0 μ Ci/ml ^{125}I . The method for measuring the efflux of each isotope was the same as that described for taurine efflux.

Solutions

The isosmotic solution (osmolality 299 mOsmoles/kg water; range 295-307 mOsmoles/kg water) contained (mM) 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 10 glucose and 10 Tris-MOPS, pH 7.4. The hyposmotic solutions were similar in composition except that they contained either 75 mM or 100 mM NaCl and had an osmolality of 186 mOsmoles/kg water (range 179-192) and 245 mOsmoles/kg water (range 230-240) respectively. The osmolality of the solutions was measured using an MicroOsmometer (Vitech Scientific LTD UK). Purinergic agonists were dissolved directly in the isosmotic and hyposmotic buffers no more than 60 min prior to use.

Statistics

Results are presented as means $\pm$ SEM. Differences were assessed using Student's paired or unpaired t-test as appropriate and were considered significant when $P < 0.05$. Inhibition of volume-sensitive taurine efflux was calculated from the areas under the curves after correction for basal efflux had been made.

Results

Effect of anion transport inhibitors on taurine release and comparison of taurine, Cl⁻ and I⁻ efflux time-courses

The first step in the investigation was to establish whether or not swelling-induced taurine efflux utilizes volume-activated chloride channels. Therefore, we 1) examined the effect of volume-activated anion channel inhibitors on taurine efflux and 2) compared the time courses of volume-activated taurine, chloride and iodide release. Reducing the osmolality of the incubation medium by 38% increased the fractional loss of taurine from both MDA-MB-231 and MCF-7 cells (Fig 1). The profile of volume-sensitive taurine efflux differed between the two cell types. Swelling-induced taurine release from MDA-MB-231 cells increased to reach a maximum value 2-3 min after the administration of the osmotic shock and declined thereafter. The fractional efflux was increased (basal-to-peak) from $0.0212 \pm 0.0073 \text{ min}^{-1}$ to $0.2142 \pm 0.0111 \text{ min}^{-1}$ ($\pm \text{SE}$, $n=3$, $P < 0.001$). Volume-activated taurine efflux from MCF-7 cells reached a maximum value 3 min after the cells were exposed to an osmotic shock and remained at or near the maximum level for the remainder of the time-course: the fractional loss of taurine (basal-to-peak) was increased from $0.0157 \pm 0.0044 \text{ min}^{-1}$ to $0.1205 \pm 0.0049 \text{ min}^{-1}$ ($\pm \text{SE}$, $n=3$, $P < 0.01$). The stilbene derivative DIDS, an established inhibitor of volume-activated anion channels, blocked volume-activated taurine efflux from both MDA-MB-231 and MCF-7 cells in a dose-dependent manner (Fig 1). DIDS at a concentration of 50 μM , 100 μM and 1000 μM respectively inhibited the volume-dependent moiety of taurine efflux from MDA-MB-231 cells by $29.4 \pm 6.2\%$, $38.3 \pm 2.9\%$ and $45.6 \pm 6.9\%$ ($\text{IC}_{50} > 1000 \mu\text{M}$). Volume-activated taurine efflux from MCF-7 cells was more sensitive to DIDS, thus, the compound at 50 μM , 100 μM and 1000 μM respectively blocked taurine efflux by $65.8 \pm 7.2\%$, $82.9 \pm 6.5\%$ and $96.1 \pm 2.6\%$ ($\text{IC}_{50} < 50 \mu\text{M}$). Taurine efflux, under isosmotic conditions, from MDA-MB-231 and MCF-7 cells was not inhibited by DIDS (Fig 1). Fig. 2 shows that diiodosalicylate, an inhibitor of volume-activated chloride channels in neutrophils [15], reduced volume-activated taurine release from MDA-MB-231 and MCF-7 cells in a dose-dependent fashion. Diiodosalicylate at a concentration of 100 μM and 1000 μM respectively reduced taurine efflux from MDA-MB-231 cells by $46.3 \pm 6.1\%$ and $91.9 \pm 1.3\%$. Taurine release from MDA-MB-231 under isosmotic conditions cells was unaffected by diiodosalicylate (Fig 2a). Volume-activated taurine

Fig. 1. The effect of a hypotonic shock (-38%) on taurine efflux from a) MDA-MB-231 and b) MCF-7 cells in the absence (■) and presence of 50 μM (□), 100 μM (○) and 1000 μM (◆) DIDS. The isosmotic buffer contained (mM) 135 NaCl, 5 KCl, 2 CaCl_2 , 1 MgSO_4 , 10 glucose and 10 Tris-MOPS, pH 7.4. The hypotonic buffer was similar in composition except that the NaCl concentration was reduced to 75mM. When required, DIDS was present throughout the entire time-course. Each point is the mean $\pm$ SE of three experiments.

release from MCF-7 cells was reduced by $77.8 \pm 1.8\%$ and $97.7 \pm 1.9\%$ respectively by 100 μM and 1000 μM diiodosalicylate. On the other hand, diiodosalicylate appeared to stimulate taurine efflux from MCF-7 cells incubated in an isosmotic buffer (Fig 2b).

The effect of a hypotonic challenge on Cl⁻ and I⁻ efflux from MDA-MB-231 and MCF-7 cells is shown in Fig 3. Reducing the osmolality of the incubation medium by 38% increased Cl⁻ and I⁻ efflux from both MDA-MB-231 ($P < 0.01$ for Cl⁻; $P < 0.02$ for I⁻) and MCF-7 ($P < 0.01$ for Cl⁻; $P < 0.001$ for I⁻) cells. The profiles of swelling-induced Cl⁻ and I⁻ efflux from MDA-MB-231 cells were similar to one another but differed from that of volume-

Fig. 2. The effect of a hypotonic shock (-38%) on taurine efflux from a) MDA-MB-231 and b) MCF-7 cells in the absence (■) and presence of 100 μ M (□) and 1000 μ M (●) diiodosalicylate. The composition of the isotonic and hypotonic buffers were the same as those described in Fig. 1. When required, diiodosalicylate was present throughout the entire time-course. Each point is the mean $\pm$ SE of 4 or 5 experiments.

activated taurine efflux. Thus, Cl⁻ and I⁻ efflux reached a maximum value 2 min after the cells were challenged and rapidly declined during the remainder of the time-course. Indeed, Cl⁻ and I⁻ efflux had almost reached a pre-stimulatory level by the end of the time-course. The profiles of volume-sensitive Cl⁻ and I⁻ efflux from MCF-7 cells were similar to one another but again were different to that of taurine efflux. Thus, swelling-induced Cl⁻ and I⁻ efflux reached a maximum value 2-3 min following the osmotic shock and thereafter, unlike taurine release, declined rapidly.

Fig. 3. The effect of a hypotonic challenge (-38%) on Cl⁻ (■) and I⁻ (□) efflux from a) MDA-MB-231 and b) MCF-7 cells. The composition of the isotonic and hypotonic buffers were the same as those described in Fig. 1. Each point is the mean $\pm$ SE of 3 experiments.

The effect of ATP on volume-activated taurine release

Extracellular ATP stimulates osmolyte efflux from a variety of cells under both isotonic and hypotonic conditions [4]. In view of this, we examined the effect of ATP on taurine release from MDA-MB-231 and MCF-7 cells. Extracellular ATP had no effect on taurine release from MDA-MB-231 cells under isotonic conditions (results not shown). In contrast, extracellular ATP stimulated the hypototically-sensitive portion of taurine efflux $P < 0.01$ (Fig 4a). Volume-activated taurine efflux

Fig. 4. a) Taurine efflux from MDA-MB-231 cells exposed to an osmotic shock (-38%) in the absence (■) and presence of 10 μ M (●) and 100 μ M (□) ATP. The composition of the isosmotic and hyposmotic buffers was the same as those described in Fig.1. Each point is the mean $\pm$ SE of 3 experiments. b) Taurine efflux from MDA-MB-231 cells exposed to a 21% hyposmotic shock in the absence (■) and presence of 100 μ M ATP (●) or 100 μ M UTP (□). The isosmotic buffer was the same as that described in Fig.1. The hyposmotic buffer was similar in composition to the isosmotic buffer except that the NaCl concentration was reduced to 100 mM. Each point is the mean $\pm$ SE of 4 experiments.

(basal-to-peak response) in the absence and presence of ATP at a concentration of 10 μ M and 100 μ M was respectively $0.1816 \pm 0.0176 \text{ min}^{-1}$, $0.3428 \pm 0.0098 \text{ min}^{-1}$ and $0.3792 \pm 0.0266 \text{ min}^{-1}$ ($\pm$ SE, $n=3$). It is apparent from Fig. 4a that extracellular ATP also increased the rate of inactivation of volume-sensitive taurine efflux from MDA-MB-231 cells. Extracellular ATP (100 μ M) also increased taurine efflux from MDA-MB-231 cells exposed to a 21% hyposmotic shock ($P < 0.01$) (Fig 4b). Swelling-induced taurine efflux (basal-to-peak response) in the absence and presence of extracellular ATP was respectively $0.0209 \pm 0.0019 \text{ min}^{-1}$ and $0.1238 \pm 0.0099 \text{ min}^{-1}$. Volume-activated taurine release from MDA-MB-231 cells was also stimulated by extracellular UTP ($P < 0.001$) at a concentration of 100 μ M ($0.0209 \pm 0.0019 \text{ min}^{-1}$ v. $0.1118 \pm 0.0063 \text{ min}^{-1}$) (Fig 4b). UTP, like ATP also enhanced the rate of inactivation of taurine efflux in the latter part of the time-course. In contrast, volume-activated taurine efflux from MCF-7 cells was largely unaffected by extracellular ATP (Fig 5). However, it was apparent that ATP reduced volume-activated taurine efflux in the latter part of the time-course.

The effect of extracellular ATP on swelling-induced taurine efflux from MDA-MB-231 cells was partially dependent upon external Ca^{2+} . Fig 6 shows the effect of both short (5min) and long term ($\sim 90 \text{ min}$) calcium

Fig. 5. Taurine efflux from MCF-7 cells exposed to a 38% hypotonic shock in the absence (■) and presence of 100 μ M ATP (□). The isosmotic and hyposmotic buffers had the same composition as those described in Fig 1. Each point is the mean $\pm$ SE of 3 experiments.

removal from the incubation buffer on taurine efflux in the presence of extracellular ATP. Calcium removal decreased the effect of extracellular ATP on volume-sensitive taurine release. It is notable that long-term calcium removal had a larger effect on the ATP-sensitive portion of efflux ($P < 0.05$). Ionomycin (1 μ M) had only a

Fig. 6. The effect of short (5 min) and long-term (~ 90 min) calcium removal on volume-activated taurine efflux in the presence of 100 μ M ATP from MDA-MB-231 cells exposed to a 21% hypotonic shock. The composition of the isosmotic buffer was the same as that described in Fig. 1. The hypotonic buffer was similar to the isosmotic solution except that the NaCl concentration was reduced to 100 mM. Values shown represent the basal-to-peak differences. Each point is the mean $\pm$ SE of 3-4 experiments.

small, albeit significant, effect on taurine release from MDA-MB-231 cells incubated in an isosmotic buffer. Thus, the fractional release was increased (basal-to-peak) from $0.0144 \pm 0.0024 \text{ min}^{-1}$ to $0.0277 \pm 0.0048 \text{ min}^{-1}$ ($\pm$ SE, $n = 4$, $P < 0.05$). Taurine release from MDA-MB-231 cells under hypotonic conditions was markedly stimulated ($P < 0.01$) by ionomycin (Fig 7a). Ionomycin, like ATP also increased the rate of inactivation of taurine efflux in the latter part of the time-course (Fig 7a). In contrast, ionomycin had no effect on volume-activated taurine efflux from MCF-7 cells (Fig 7b).

Suramin inhibited ($P < 0.001$) the effect of ATP on swelling-induced taurine efflux from MDA-MB-231 cells (Fig 8a). Note, however, that suramin also inhibited ($P < 0.01$) volume-activated taurine release from MDA-MB-231 cells in the absence of exogenously added extracellular ATP. In this connection, suramin inhibited ($P < 0.001$) volume-activated taurine release from MCF-7 cells (Fig 8b). Suramin also inhibited taurine efflux from MCF-7 cells under isosmotic conditions ($P < 0.01$).

The effect of adenosine on taurine efflux

Extracellular adenosine (100 μ M) stimulated taurine efflux from MDA-MB-231 cells which had been exposed to either a 38% or 21% osmotic shock (Fig 9). Adenosine increased the fractional efflux (peak response) from

Fig. 7. a) The effect of a hypotonic shock on taurine efflux from MDA-MB-231 cells in the absence (■) and presence (●) of ionomycin (1 μ M). The points denoted (□) represent the effect of ionomycin (1 μ M) on taurine efflux from MDA-MB-231 cells incubated in an isosmotic buffer. The composition of the buffers were the same as those described in Fig 1 except that all buffers also contained 0.1% DMSO. Each point is the mean $\pm$ SE of 4-5 experiments. b) the effect of a hypotonic shock on taurine efflux from MCF-7 cells in the absence (■) and presence (□) of ionomycin (1 μ M). The composition of the buffers was the same as those described in Fig 1 except that all buffers also contained 0.1% DMSO. Each point is the mean $\pm$ SE of 4 experiments.

$0.1993 \pm 0.0158 \text{ min}^{-1}$ to $0.3247 \pm 0.0392 \text{ min}^{-1}$ ($\pm$ SE, $n = 4$, $P < 0.05$) when the cells were exposed to a 38% osmotic challenge (Fig 9a). The fractional efflux of taurine (peak response) from cells exposed to a 21% osmotic shock was increased from 0.0412 ± 0.0028

Fig. 8. a) the effect of suramin (0.5 mM) on taurine efflux from MDA-MB-231 cells exposed to a 38% hypotonic shock in the absence (■, + suramin ●) and presence (□, + suramin ◆) of extracellular ATP (100 μM). The composition of the buffers were the same as those described in Fig 1. Each point is the mean ± SE of 3 experiments. b) taurine efflux from MCF-7 cells exposed to a 38% hypotonic shock in the absence (■) and presence (□) of suramin (0.5 mM). Each point is the mean ± SE of 4 experiments.

Fig. 9. a) The effect of a 38% reduction in the external osmolality on taurine release from MDA-MB-231 cells in the absence (■) and presence (□) of adenosine (100 μM). The composition of the buffers is the same as those described in Fig 1. B) the effect of a 21% osmotic shock on taurine release from MDA-MB-231 cells in the absence (■) and presence (□) of adenosine (100 μM). The composition of the buffers is the same as those described in Fig. 5b. Each point is the mean ± SE of 4 experiments.

min⁻¹ to 0.0917 ± 0.0111 min⁻¹ (± SE, n=4, $P < 0.05$) by the addition of adenosine (Fig 9b).

Discussion

Relationship between swelling-induced taurine and chloride efflux

It has been suggested that swelling-activated taurine efflux utilizes volume-sensitive chloride channels. This notion is based primarily on the effect of pharmacological

agents and to a lesser extent on electrophysiological evidence. The finding that volume-activated taurine efflux is inhibited by chloride channel blockers such as DIDS and NPPB was initially taken as evidence for a common pathway [5, 16]. Whole-cell patch clamp studies have also shown that taurine efflux is able to permeate chloride channels [6,17-19]. The present finding that swelling-induced taurine efflux from both MDA-MB-231 and MCF-7 cells is inhibited by chloride channel blockers such as DIDS and diiodosalicylate does constitute *prima facie* evidence for a shared pathway. However, chloride channel

blockers are non-specific. For example, DIDS is known to block anion-exchangers and K^+ channels as well as volume activated Cl^- channels [20, 21].

On the other hand, we found that volume-activated taurine efflux from MDA-MB-231 and MCF-7 cells had a markedly different time course from that of swelling-induced Cl^- and I^- efflux suggesting that taurine and Cl^- are exiting via separate pathways. Similar time courses would have been expected if chloride and taurine shared a common pathway. Volume-activated Cl^- and I^- efflux inactivated at a much faster rate than taurine efflux from MDA-MB-231 cells. Similarly, swelling-induced Cl^- and I^- efflux from MCF-7 cells decreased with time whilst volume-activated taurine efflux was maintained. Stutzin et al [22] have shown that volume-activated chloride and taurine efflux from HeLa cells have different time courses and therefore concluded that they do not share a common pathway. Other studies have suggested that volume-sensitive taurine efflux does not always utilize anion channels. It is apparent that taurine efflux in Ehrlich Ascites cells [7], lactating rat mammary tissue [8, 9], skate erythrocytes [23], supraoptic glial cells [24], C6 glioma cells [25] and cultured human intestinal epithelial cells [26] is independent from volume-activated chloride channels. It is also claimed that volume-activated taurine efflux from some cell types is via an anion exchanger [27].

The finding that DIDS had a much greater effect on taurine release from MCF-7 cells than MDA-MB-231 cells suggests that latter expresses more than one route for volume-activated taurine efflux: one which is sensitive to DIDS and one or more which is not inhibited by the stilbene derivative. In relation to this, it has been reported that swelling-induced taurine release from mouse blastocysts and intestinal 407 cells is not inhibited by DIDS [26, 28]. Recent evidence suggests that Caco-2 cells express more than one volume-activated taurine efflux pathway [29]. It is apparent that Caco-2 cells possess a tamoxifen-sensitive, volume-activated taurine efflux pathway on the apical membrane and a tamoxifen-insensitive, volume-activated, taurine release pathway on the basolateral membrane.

The regulation of taurine efflux by extracellular ATP and adenosine

Extracellular ATP has been shown to modulate volume-activated transport processes, including taurine efflux, from a variety of cell types although the effect is by no means uniform [4]. Several groups have studied the association between extracellular ATP and volume-sensitive taurine efflux and the relationship appears to be

complex and dependent upon cell type: some studies have reported a stimulation of taurine release by ATP [19, 30, 31], some have found that ATP inhibits taurine efflux [24] whereas others have found no effect [26]. The present study shows that MDA-MB-231 and MCF-7 cells differed in their response to extracellular ATP. Volume-sensitive taurine release from MDA-MB-231 cells was stimulated by ATP whereas taurine efflux from MCF-7 cells was unaffected. The lack of an effect of extracellular ATP on taurine release from MCF-7 cells is surprising given that MCF-7 cells appear to express P2 receptors and that I^- efflux can be stimulated by ATP under isosmotic conditions [32]. However, in our hands, extracellular ATP had no effect on I^- efflux from MCF-7 cells incubated in an isosmotic or a hyposmotic buffer (Shennan & Thomson, unpublished observations). In addition, we have recently shown that swelling-induced K^+ efflux from MCF-7 cells is unaffected by ATP even though the intracellular $[Ca^{2+}]$ increased [13].

The finding that UTP had a similar effect to that of ATP on taurine efflux from MDA-MB-231 cells coupled with the observation that ATP-induced taurine efflux was inhibited by suramin suggests that ATP may be acting via P2, possibly P2Y2, receptors. It is envisaged that in this breast cancer cell line ATP stimulates Ca^{2+} uptake as well as Ca^{2+} release from intracellular stores [13]. However, several lines of evidence suggest that a rise in intracellular Ca^{2+} *per se* is not responsible for stimulating volume-activated taurine efflux. Firstly, taurine efflux from MDA-MB-231 cells, under isosmotic conditions, was not affected by extracellular ATP despite the fact that ATP has been shown to increase intracellular Ca^{2+} [13] and secondly, ionomycin under isosmotic conditions did not mimic the effect of ATP on taurine efflux from MDA-MB-231 cells. In contrast, ionomycin had a similar effect to ATP on taurine efflux from MDA-MB-231 cells measured under hyposmotic conditions. It appears that swelling-induced taurine release has to be activated before extracellular ATP can exert its effect on taurine efflux. It is possible that extracellular ATP makes volume-activated taurine efflux from MDA-MB-231 cells more sensitive to an osmotic shock. A similar conclusion was recently reached by Franco et al [31] who studied the effect of ATP on taurine efflux from Swiss 3T3 fibroblasts.

ATP and UTP enhanced the inactivation rate of swelling-induced taurine efflux from MDA-MB-231 cells in the latter stages of the time-course. This may be related to the effect of ATP and UTP on K^+ efflux and, by inference, on cell volume regulation. Gow et al [13] have recently shown that K^+ release from MDA-MB-231 cells

is stimulated by extracellular ATP under both isosmotic and hyposmotic conditions. It is predicted that ATP and UTP will stimulate the rate of volume regulation in MDA-MB-231 cells which in turn abrogates the need for volume-activated taurine efflux [33]. Alternatively, the inactivation of taurine efflux may be an inherent property of the transport pathway.

We found that suramin inhibited volume-activated taurine efflux from MDA-MB-231 cells even in the absence of exogenously added ATP. In addition, suramin blocked volume-sensitive taurine efflux from MCF-7 cells. There are at least two explanations for this. Suramin could be acting directly on the volume-activated pathway to inhibit taurine efflux and not necessarily via P2 receptors [19]. Alternatively, suramin could be inhibiting the effect of ATP released from cultured human breast cancer cells as a consequence of cell swelling. It is well documented that ATP released from cells following cell swelling is able to activate membrane transport pathways (eg see [34]).

The results show that adenosine stimulated volume-sensitive taurine efflux from MDA-MB-231 cells. This is consistent with the recent report by Panjehpour et al [35] which describes the presence of functional A_{2B} adenosine receptors in MDA-MB-231 cells. The finding that extracellular adenosine was not as effective as ATP at stimulating taurine release suggests that the effect of ATP on taurine efflux is not due to hydrolysis of ATP to adenosine. Furthermore, it is unlikely that adenosine is acting via the ATP receptor. It would appear, therefore, that the control of volume-activated taurine efflux in MDA-MB-231 cells by purines involves more than one type of receptor.

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