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Small-cell Lung Cancer (Human): Potentiation of Endocytic Membrane Activity by Voltage-gated Na^+ Channel Expression in Vitro

P.U. Onganer, M.B.A. Djamgoz
Neuroscience Solutions to Cancer Research Group, Department of Biological Sciences, Sir Alexander Fleming Building, Imperial College London, South Kensington Campus, London SW7 2AZ, UK

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Abstract. The possible functional role of voltage-gated Na^+ channel (VGSC) expression in controlling endocytic membrane activity in human small-cell lung cancer (SCLC) cell lines (H69, H209, H510) was studied using uptake of horseradish peroxidase (HRP). The normal human airway epithelial (16HBE14o) cell line was used in a comparative approach. Uptake of HRP was vesicular, strongly temperature-sensitive and suppressed by cytoskeletal poisons (cytochalasin D and colchicine), consistent with endocytosis. Compared with the normal cells, HRP uptake into SCLC cells was kinetically more efficient, resulting in more than four-fold higher uptake under optimized conditions. Importantly, HRP uptake into SCLC cells was inhibited significantly by the specific VGSC blocker tetrodotoxin, as well as lidocaine and phenytoin. These effects were dose-dependent. None of these drugs had any effect on the uptake into the 16HBE14o cells. Uptake of HRP into SCLC cells was reduced by ~66% in Na^+-free medium and was partially (~30%) dependent on extracellular Ca^{2+}. The possibility that the endocytic activity in the H510 SCLC cells involved an endogenous cholinergic system was investigated by testing the effects of carbachol (a cholinergic receptor agonist) and eserine (an inhibitor of acetylcholinesterase). Both drugs inhibited HRP uptake, thereby suggesting that basal cholinergic activity occurred. It is concluded that VGSC upregulation could enhance metastatic cell behavior in SCLC by enhancing endocytic membrane activity.

Key words: Voltage-gated sodium channel — Tetrodotoxin — Phenytoin — Lidocaine — Small-cell lung cancer — Endocytosis

Correspondence to: M. Djamgoz; email: m.djamgoz@imperial.ac.uk

Introduction

Small-cell lung cancer (SCLC) is one of the most aggressive forms of cancer, which develops very rapidly and will frequently have metastasized by the time of primary diagnosis (Ihde et al., 1995). Cytotoxic chemotherapy is the main method for treating metastatic human SCLC but rarely produces a permanent cure. There is an urgent need, therefore, to develop new, early diagnostic markers and therapeutic methods for SCLC. It has been shown previously that metastatic cells of human origin express high levels of voltage-gated Na^+ channels (VGSCs) in prostate cancer (Laniado et al., 1997), breast cancer (Fraser et al., 2002; Roger, Besson & LeGuennec, 2003) and melanoma (Allen, Lepple-Wienhues & Cahalan, 1997). In the human genome, ten different VGSC α-subunits have been found (Plummer & Meisler, 1999). These can broadly be divided into two groups, depending upon the concentration of tetrodotoxin (TTX) needed for blockage. Thus, TTX-sensitive (TTX-S) and TTX-resistant (TTX-R) VGSCs are blocked by nM and μM TTX, respectively (Goldin, 2001; Waxman, 2000).

The role of the VGSCs in metastatic cell behavior has been studied extensively in prostate cancer (rat and human). The highly specific VGSC blocker TTX suppressed (by 35–100%) a range of cellular behavior involved in the metastatic cascade, including process extension (Fraser et al., 1999), directional motility (Djamgoz et al., 2001; Fraser et al., 2003), secretory membrane activity / endocytosis (Mycielska et al., 2003; Krasowska et al., 2004), adhesion (Mycielska & Djamgoz, 2004) and invasiveness (Grimes et al., 1995; Laniado et al., 1997; Smith et al., 1998; Fraser et al., 2002). Importantly, also Bennett, Smith and Harper (2004) have shown that VGSC expression was "necessary

and sufficient'' for the invasiveness of human prostate cancer cells. Furthermore, Anderson et al. (2003) have suggested that VGSC-blocking drugs may be useful as cytostatic inhibitors of androgen-insensitive human prostate cancer.

Interestingly, SCLC cells also have a number of marked 'neuronal / neuroendocrine' characteristics (Onganer et al., 2005). In fact, this is the most commonly encountered form of neoplasm associated with a wide range of paraneoplastic neurological disorders (Chan, Vernino & Lennon, 2001). A high level of VGSC expression and electrophysiological activity has also been found to occur in human SCLC cell lines (Blandino et al., 1995). These VGSCs were activated by membrane depolarization and blocked by TTX in a concentration-dependent manner (Blandino et al., 1995). However, the functional role of the VGSCs in progression of SCLC is not known. In the present study, we have questioned whether VGSC activity could play a role in endocytosis, an integral component of the metastatic cascade (for review *see* Floyd & De Camilli, 1998). We used uptake of horseradish peroxidase (HRP), a non-cytotoxic tracer; this method has been used extensively to measure endocytic membrane activity (e.g., Theodosis et al., 1976; Mycielska et al., 2003). Experiments were carried out on three human SCLC cell lines and a ''normal'' airway epithelial cell line, in a comparative approach.

Materials and Methods

Cell Culture

Four human cell lines were used, three SCLC (H69, H209, H510) and one normal airway epithelial (16HBE14o). The SCLC cell lines were maintained in RPMI medium, whilst the 16HBE14o cell line was maintained in Minimum Essential Medium Eagle (MEME). Both media were supplemented with 10% fetal calf serum (FCS) and 1% L-glutamine, in a humidified 37°C incubator with 5% CO_2.

Endocytosis Assay

Given SCLC cells were counted and placed in Eppendorf tubes at a density of approximately 10×10^4 cells/tube in normal tissue culture medium (300 μl). Before the start of the endocytosis assay, the cells were spun at 1000 rpm for 2 min and then the medium was replaced by the same volume of mammalian Krebs' solution (*see* next section) and left to equilibrate for 10–15 min. The solution was then replaced by 100 μl of Krebs' solution containing HRP (type IV; Sigma). In those experiments in which the ionic composition of the incubating solution was changed or a drug was added, a control with normal solution was always present. The final concentrations of HRP used ranged from 0.1 to 1 mg/ml and incubation time from 10 min to 2 h. During all experimental treatments, cells were kept sterile in the incubator at 37°C, except for the experiments at 4°C when the cells were kept in a cold room. After the incubation, the cells were rinsed three tunes with corresponding solution to remove all extracellular HRP. In order to 'release' the HRP contents of the cells, 120 μl of lysis buffer (*see* below) was added to the cell pellet. Immediately afterwards, diaminobenzidine (DAB; 0.5 mg/ml) and hydrogen peroxide (0.01%) in 120 μl of 1 M Tris buffer (pH 7.4) were added and the reaction solutions (200 μl of & each) were transferred to a 96-well plate. Importantly, the cells were spin at 1000 rpm for 2 min before each solution change, in order not to lose any cells, since SCLC cells grow in suspension. The density of the color reaction was measured at 540 nm on a plate reader. These readings were plotted as ''OD_{540}''. Where the differences between respective exogeneous (uptake) and endogenous HRP *OD*540 values were calculated, as in dose-response data, the difference was assumed to represent ''net endocytosis'' and termed ''*E*540''. In pharmacological experiments, the effect of a given drug was expressed as the percentage of the control difference value treated as 100%.

On the other hand, the 16HBE14o cells were adherent and were seeded in 24-well plates at a density of approximately 10×10^4 cells/well in normal tissue culture medium. After 24 h of incubation, the same experimental procedures, as above, were applied, without the need for spinning at every stage.

In all the experiments carried out, as above, endogenous HRP was also measured in cells processed in the same way but with no added HRP. All three sets of conditions (normal, ionic substitution/drug-added and HRP-omitted) in every experiment were tested on given cells 'side by side'. Cell viability was monitored using trypan blue staining.

Solutions

The normal Krebs' solution contained in mM: 144 NaCl, 5.4 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 5.6 D-glucose, 5 HEPES (pH 7.4, adjusted with HCl). In the ''Ca^{2+}-free'' solution, $CaCl_2$ was replaced by HEPES (increased to 10 mM) and 0.2 mM EGTA was added. In the ''Na^+-free'' solution, NaCl was replaced by equimolar choline chloride. The ''lysis buffer'' was made by (i) serially adding 0.9 g NaCl, 10 ml 10% NP-40 and 2.5 ml 10% Na-deoxycholate to a solution of 0.79 g Tris base in 75 ml distilled water and (ii) adding 1 ml of 100 mM EDTA and completing the volume to 100 ml with distilled water.

Pharmacological Agents

The following pharmacological agents were used: Tetrodotoxin (TTX), lidocaine, phenytoin, carbachol, eserine, cytochalasin D and colchicine. All these were purchased from Sigma, except TTX (Alomone).

Polymerase Chain Reaction

Reverse-transcriptase polymerase chain reaction (RT-PCR) methods were used as described by Diss et al. (2001). The expressions of the following human VGSC α-subunits were tested: Nav1.3, 1.5, 1.6, 1.7, 1.8 and 1.9. Human cytochrome b-reductase (hCyt-bR) was used as an internal control (Diss et al., 2001).

Data Analysis

Each experiment in a given condition was carried out in triplicate and the mean was determined as the representative result. Each condition was thus tested in at least 3 separate experiments. The averages and the standard errors were calculated for the experimental values and analyzed statistically by Mann-Whitney *U*-tests. Slopes of linear regressions were analyzed by *t*-test.

Results

Initial Observations

VGSC α-Subunit mRNAs Expressed

The RT-PCR studies suggested the expression of several subtypes of VGSC α-subunits in the SCLC cell lines adopted (H69, H209, H510). Nav 1.3, 1.5 and 1.6 mRNAs were present in all three cell lines. Nav 1.9 was found only in H510 cells, whereas none of the cell lines expressed Nav 1.8. In conclusion, TTX-S and TTX-R subtypes of VGSC α-subunits were co-expressed in all SCLC cell lines studied.

Optimization of HRP Uptake Protocol

Uptake of HRP into vesicles was observed in all cell lines under control conditions. In the main calorimetric assays, the uptake of HRP was time- and concentration-dependent in both SCLC (H510) and normal airway epithelial cells (Fig. 1). The time dependence showed that there was a rapid increase in HRP (0.5 mg/ml) uptake in the first 40–60 min of the incubation for both H510 and 16HBE14o cell lines (Fig. 1*A*). Longer incubation times (up to 120 min) resulted in much smaller but still significant increases for both cell lines ($P = 0.03$ and 0.002 for 120 min and 40 min, respectively). The concentration dependence showed that uptake of HRP in both cell lines (40 min of incubation) was steadily greater as the concentration was increased in the range 0.1 to 1 mg/ml (Fig. 1*B*). The values of E_{540} for the mid-range (0.5 mg/ml) were significantly different relative to respective lower and higher concentrations in both cell lines ($P < 0.003$). In conclusion, an HRP concentration of 0.5 mg/ml and an incubation period of 40 min were adopted as optimal for both cell lines in the remainder of the experiments. Under these optimal control conditions, HRP uptake into H510 cells was 4.2-fold higher compared with 16HBE14o cells ($P = 0.002$). The levels of endogenous peroxidase in the two cell types were comparable and represented 9 % and 56 % of the respective uptakes (Fig. 1*C*).

Endocytosis Control Test and Cytoskeletal Dependence

The uptake of HRP being endocytic, rather than passive, was confirmed. First, by microscopic examination of the cells (as described by Mycielska et al., 2003), uptake could be seen to be vesicular. Second, lowering the temperature of the incubation from 37°C to 4°C resulted in about 85% reduction in the HRP uptake into both cell lines; these changes were highly significant ($P < 0.001$ for both) (Fig. 2*A*). In fact, HRP uptake at 4°C was only slightly (8–15 %) higher than the respective levels of endogenous peroxidase. Finally, the sensitivity of the HRP uptake to cytoskeletal drugs was tested on H510 cells (Fig. 2*B*).

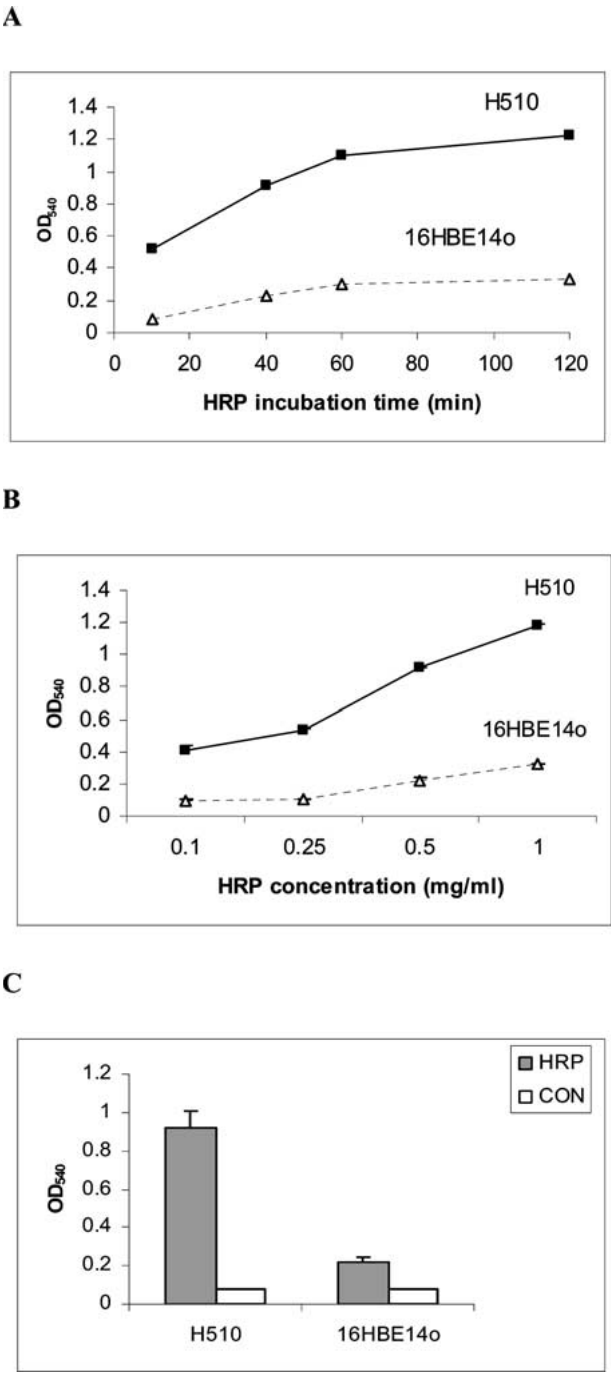


Fig. 1. Basic characteristics of HRP uptake into the human H510 SCLC cell line and 16HBE14o normal airway epithelial cell line. Time (*A*) and concentration (*B*) dependence of the HRP uptake. (*C*) Quantitative uptake of HRP under optimal conditions (0.5 mg/ml for 40 mm); "CON" indicates endogenous peroxidase activity. Each data point represents the mean ± SE ($n > 3$). In most experiments, SE values were too small to be seen above the symbols. OD_{540} denotes the quantity of exogenous HRP uptake (*see* Materials and Methods for further details).

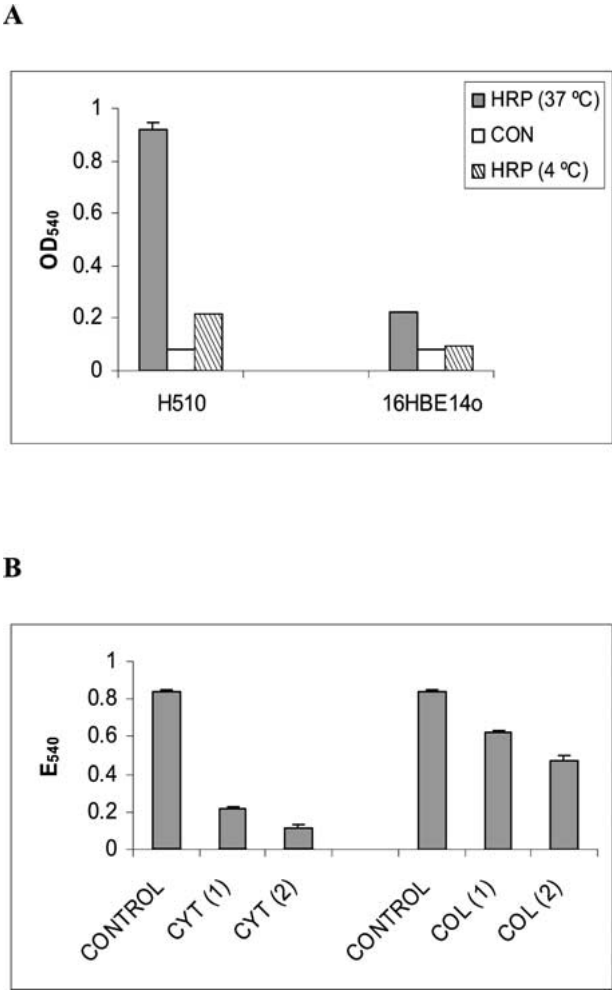


Fig. 2. Effects of lowering the incubation temperature and applying cytoskeletal poisons on optimal HRP uptake into H510 and 16HBE14o cells. (*A*) Quantitative comparison of the exogenous HRP uptake at 37°C and 4°C; "CON" indicates endogenous peroxidase activity in the two cell lines. (*B*) Effects of two different concentrations each of cytochalasin (CYT) and colchicine (COL) on H510 SCLC cells. CYT(1) and CYT(2), 2 and 5 μM cytochalasin, respectively. COL(1) and COL(2), 5 and 10 μM colchicines, respectively. E_{540} denotes the difference between total HRP (including the uptake) and endogenous HRP, i.e., "net endocytosis" (*see* Materials and Methods for further details). "CONTROL" denotes net endocytosis under non-drug-treated conditions.

Uptake was suppressed by both drugs in a concentration-dependent manner. The percentage reductions were 86% and 43% for cytochalasin D (5 μM) and colchicine (10 μM). These effects were highly significant ($P = 0.001$ and 0.002, respectively).

Endocytic Activities of SCLC Versus Normal Airway Epithelial Cell

As already noted, HRP uptake into H510 cells was more than four-fold higher compared with normal airway epithelial cells (Figs. 1*C* and 3*A*). Two other SCLC cell lines, H69 and H209, tested also gave much higher values of OD_{540} for HRP uptake compared with the normal cells (Fig. 3*A*). As regards both time and concentration dependence, the final extent (corresponding to 120 and 1 mg/ml, respectively) of the HRP uptake was significantly higher in H510 compared with 16HBE14o cells ($P = 0.001$ for both; Fig. 1*A* and *B*). In the concentration dependence, the gradient of the linear portion of the HRP uptake was also significantly steeper for H510 compared with 16HBE14o cells. In conclusion, the endocytic activities of SCLC cells were significantly higher and more efficient than the normal airway epithelial cell line.

Effects of TTX, Phenytoin and Lidocaine

The effects of the specific VGSC blocker TTX on HRP uptake into all 4 cell lines were tested (Fig. 3*A*). At 100 nM, TTX reduced HRP uptake into the SCLC cells by 80 to 89% ($P < 0.001$), close to the level of the corresponding negative control. The effect of TTX was dose-dependent in the concentration range 5 to 100 nM (Fig. 3*B*). In contrast, TTX (up to 5 μM) had no effect on the uptake into 16HBE14o cells (Fig. 3*A* and *B*). In the remainder of the experiments, mainly the H510 cell line was used as representative of SCLC.

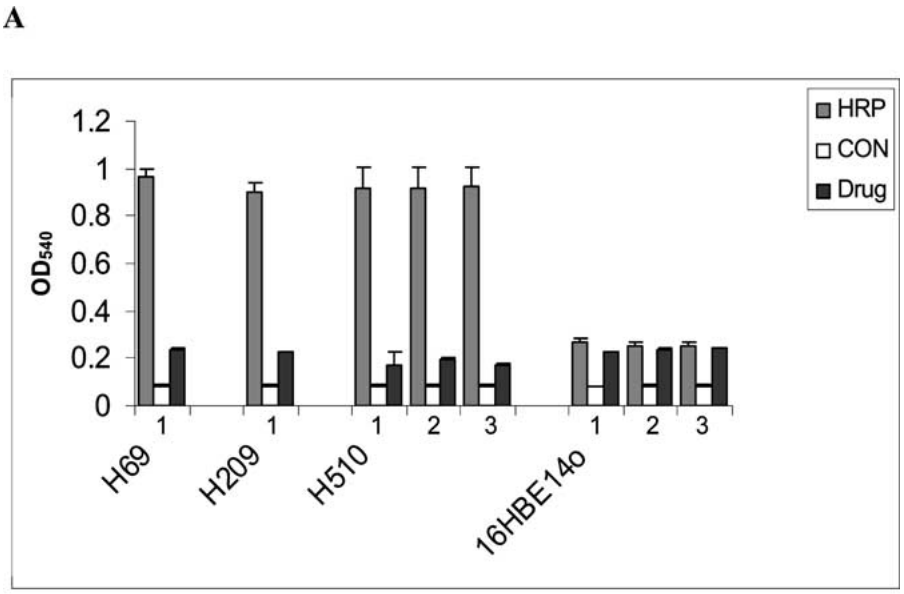
Two other VGSC blocker drugs, lidocaine (local anaesthetic) and phenytoin (anticonvulsant) were also tested. Lidocaine and phenytoin (both 200 μM) also decreased HRP uptake into H510 cells by 86% and 88%, respectively ($P = 0.001$ for both) (Fig. 3*A*). These effects were concentration-dependent (Fig. 3*C*). In contrast, again, there was no effect on the uptake into the 'normal' 16HBE14o cells (Fig. 3*A*).

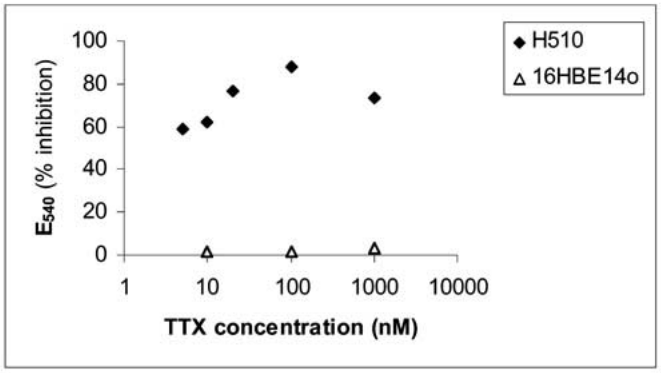
Ionic Dependence of Endocytosis

Uptake of HRP into H510 cells was dependent, at least partially, upon extracellular Na^+ and Ca^{2+} concentrations (Fig. 4). Thus, in Na^+-free medium, uptake was reduced by 66% ($P < 0.001$). Removal and chelation of extracellular Ca^{2+} also reduced the uptake (by about 30%; $P < 0.05$) (Fig. 4).

Effects of Cholinergic Drugs

Since the cholinergic system is important in SCLC and acetylcholine has been found to be synthesized and released as a trophic factor (e.g., Song et al., 2003), we questioned whether a cholinergic effect might be involved in the endoytosis by challenging the HRP uptake with two different cholinergic drugs. Thus, the effects of co-treatment of H510 cells with carbachol (a cholinergic receptor agonist) and eserine





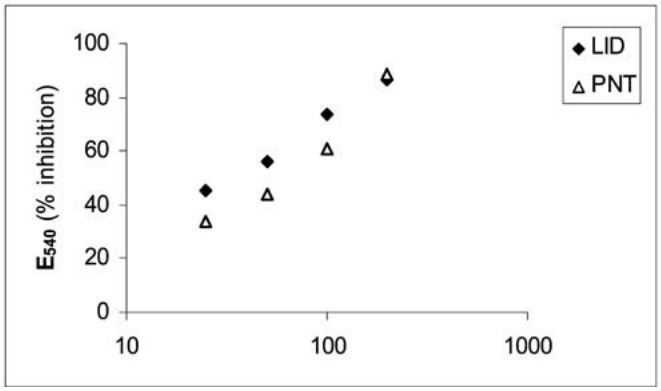


Fig. 3. Inhibition of endocytosis by tetrodotoxin (TTX), lidocaine (LID) and phenytoin (PNT) on three SCLC cell lines (H69, H209, H510) and normal airway epithelial cell line (16HBE14o) and their dose dependence. (*A*) Each data set of 3 histobars denote the following (left to right): (1) Gray bars (*HRP*), optimal uptake under non-drug-treated control conditions; white bars (*CON*), endogenous peroxidase activity; and black bars (*Drug*), 100 nM TTX. (2) As in (1), but for 200 μM lidocaine as *Drug*. (3) As in (1), but for 200 μM phenytoin as *Drug*. (*B*) Dose dependence of the effect of TTX in the concentration range 5 to 1000 nM on HRP uptake expressed as defined in the Materials and Methods. Data for H510 and 16HBE14o cells are indicated by diamonds and triangles, respectively. (*C*) Dose dependence of the effects of lidocaine (*LID*; diamonds) and phenytoin (*PNT*; triangles) in the concentration range 25 to 200 μM (data plotted as in (*B*)). Each data point represents the mean ± SE ($n > 5$). In most experiments, SE values were too small to be seen above the symbols.

(an acetylcholinesterase inhibitor) during HRP uptake were tested. Both drugs (at 1 μM) caused a decrease in HRP uptake, by 18% and 80% respectively ($P < 0.05$ for both; Fig. 5). In conclusion, cholinergic effects could indeed be involved in the endocytic activity studied.

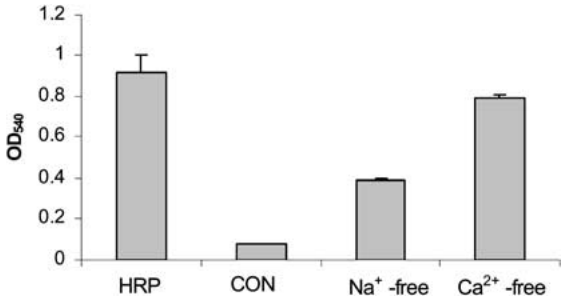


Fig. 4. Ionic dependence of exogenous HRP uptake into H510 cells under the optimal experimental conditions. "HRP" and "CON" are as defined in Figure 1. In the "Na^+-free" solution, all external NaCl was replaced by equimolar choline chloride. In the "Ca^{2+}-free", solution, all external $CaCl_2$ was removed and EGTA added. Each histobar represents the mean ± SE ($n > 10$). In most experiments, SE values were too small to be seen above the averages.

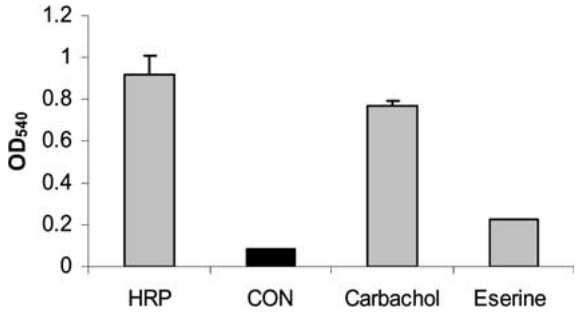


Fig. 5. Effects of cholinergic drugs (carbachol and eserine, both 1 μM) on optimal HRP uptake into H510 cells. Uptake was quantified as OD_{540}, as defined in Figure 1 legend. "HRP" and "CON" (both relating to non-drug-treated condition) are as defined in Figure 1 legend. Each data point represents the mean ± SE ($n > 3$). In most experiments, SE values were too small to be seen above the averages.

Discussion

The main conclusions of this study are as follows: 1) Although both SCLC and normal airway epithelial cells possess basal endocytic membrane activity, as measured by HRP uptake, activity was much higher in cancer cells. (2) Endocytosis in SCLC (but not normal epithelial) cells was inhibited by blockers of VGSCs known to be expressed in these cells. (3) Endocytosis was partially dependent upon extracellular Na^+ and Ca^{2+}. (4) Endocytosis could involve the cholinergic system known to operate in SCLC.

Measurement of Endocytosis by HRP Uptake

In this study, uptake of HRP was used as a non-cytotoxic tracer of endocytic membrane activity. Similar methods were used previously to measure endocytosis in a variety of cell types, including amphibian photoreceptor synaptic terminals (Schacher, Holtzman & Hood, 1976), pancreatic acinar ells (Freedman et al., 1999), corneal endothelial cells (Gordon et al., 1998) and rat prostate cancer cells (Mycielska et al., 2003; Krasowska et al., 2004). Lowering the experimental temperature significantly reduced uptake, consistent with it representing endocytosis. Furthermore, two cytoskeletal toxins (cytochalasin D and colchicine) also were suppressive, consistent with the uptake involving vesicular trafficking, observed earlier by microscopy. Previous results also reported that endocytosis would depend on microfilament and microtubule components of the cytoskeleton (Cole, Villa & Wilkinson, 2000; Okamoto et al., 2001; Mycielska et al., 2003). The slopes of the HRP uptake were significantly greater for SCLC compared with normal cells as regards concentration but not time dependence (Figs. 1*A* and *B*). The latter possibly reflects the complex dynamics of endocytosis involving both slow and fast components (Gruenberg, 2001; Mycielska et al., 2003). Nevertheless, optimized uptake was significantly higher for SCLC versus normal airway epithelial cells (Figs. 1*A* and *B*).

Potentiation of Endocytosis by VGSC Expression/Activity

All three SCLC cell lines studied were found to be much more endocytic than normal airway epithelial cells. These results agree generally with earlier observations showing similarly that strongly metastatic rat prostate and human breast cancer cell lines are much more endocytic, compared with the weakly metastatic counterparts (Fraser et al., 2002; Mycielska et al., 2003). Treatment of the SCLC cells with TTX significantly reduced the endocytosis, again, as found earlier for prostate and breast cancer cells (Fraser et al., 2002; Mycielska et al., 2003). In contrast, TTX had no effect on normal or weakly metastatic cells. These results are consistent, first, with VGSC expression occurring selectively in strongly metastatic carcinoma cell lines (Blandino et al., 1995; Grimes et al., 1995; Laniado et al., 1997); second, in all three carcinomas studied, VGSC activity enhanced endocytosis, an integral part of the metastatic cascade.

A previous electrophysiological study reported that an SCLC cell (H146) studied expressed mainly TTX-R VGSCs (Blandino et al., 1995). In the present study, both TTX-R and TTX-S VGSC mRNAs were found to be expressed in all three SCLC cell lines studied. Multiplicity of VGSC expression has been seen in other cell types before (Diss, Fraser & Djamgoz, 2004). The difference seen in the SCLC cells could be due to different cell lines and/or techniques

being employed. Nevertheless, the concentration dependence of the effect of TTX on endocytosis suggested that it was TTX-S VGSC activity that controlled endocytosis. It is possible that other components of metastasis are controlled by different VGSC subtype(s).

Ionic Control of Endocytosis

In H510 cells, when Na^+ was removed from the incubation solution, significant decrease of HRP uptake occurred. This result is consistent with the TTX data and together they suggest that endocytic activity in strongly metastatic SCLC cell line H510 is enhanced by functional VGSC expression. In the absence of Ca^{2+}, only a small reduction in HRP uptake in SCLC cell line H510 was found, suggesting that endocytosis was mainly Ca^{2+}-independent. Endo/exocytosis independent of extracellular Ca^{2+} has been found to occur in other cells (Oda et al., 1999, Cousin, 2000). At present, the precise mechanism(s) underlying the VGSC control of endocytosis is not known. Various possibilities exist, as discussed by Mycielska et al. (2003), and include vesicular mobility resulting from Ca^{2+} released from intracellular stores as a result of Na^+-induced local pH change (Willoughby, Thomas & Schwiening, 2001) and/or protein kinase A activation by intracellular Na^+ (Hilfiker et al., 2001). Further work is required to evaluate these possibilities.

Apparent Cholinergic Involvement in Endo/Exocytosis

Endocytosis serves many important cellular functions including uptake of extracellular nutrients, regulation of cell-surface protein expression, maintenance of cell polarity and antigen presentation (Mukherjees, Ghosh & Maxfield, 1997). Importantly, also, endocytosis mirrors exocytosis (Smith et al., 2000; Kilic et al., 2001) and can be used as a measure of cells' secretory activity (Mycielska et al., 2003). Multiple reports have shown that SCLC cells express nicotinic and muscarinic acetycholine receptors (Cunningham et al., 1985; Maneckjee & Minna, 1990; Williams & Lennon, 1990 ; Tarroni et al., 1992) and that their activation with acetycholine (ACh) (Schuller et al., 1990), nicotine (Fucile, Napolitano & Mateei, et al., 1990; Schuller et al., 1990, Quik, Chan & Patrick, 1994) or muscarine (Fucile et al.1990; Schuller et al. 1990) stimulates the growth of SCLC cells. In fact, SCLC cells have been shown to synthesize and release ACh (Song et al., 2003). We probed this question in H510 cells by investigating the effects of perturbing the balance of cholinergic activity by two different drugs, the cholinergic receptor agonist carbachol and the acetylcholinesterase (AChE) inhibitor, eserine. Both treatments, especially AChE inhibition, caused significant decreases in the HRP uptake. Thus, presumably, prolonging the lifetime and availability of endogeneous ACh released, by inhibiting AChE, would slow down the secretory process. This is consistent with SCLC cells using ACh in an autocrine loop to regulate growth (Song et al., 2003). SCLC cells also release a number of other compounds, including growth factors such as gastrine-releasing peptide (Oremek & Sapoutzis, 2003), important to the metastatic process (Moody et al., 2003). It will be interesting to determine in future studies whether VGSC activity controlled their specific secretions.

Possible Clinical Relevance

Voltage-gated Na^+ channels are already targets of numerous clinical drugs (Chen et al., 2000; Clare et al., 2000; O'Leary & Chahine, 2002; Wang & Wang, 2003). A widely used local anaesthetic and class I anti-arrhythmic, lidocaine, suppresses VGSC activity in a use-and voltage-dependent way (Ragsdale et al., 1994; Kondratiev & Tomaselli, 2003). Phenytoin (or Diphenylhydantom) is another clinically useful anticonvulsant/class 1b antiarrhythmic and also suppresses VGSC activity (Barber, Starmet & Grant, et al. 1991; Tunnicliff, 1996; McCleane, 1999; Backonja, 2002). Both drugs were found to decrease the HRP uptake into SCLC cells, whilst having no effect on the normal airway epithelial cells. Phenytoin was also shown earlier to suppress release of prostate-specific antigen (PSA) from human prostate cancer *in vitro* (Abdul & Hoosein, 2001).

In conclusion, VGSC expression/activity enhances endocytosis, and possibly secretory membrane activity, in SCLC cells. VGSC activity has been shown previously to potentiate a variety of other cellular behaviors involved in the metastatic cascade in prostate and breast cancer. It would appear, therefore, VGSC upregulation may be a general mechanism that increases metastatic potential and could serve as a novel target in the clinical management of carcinomas.

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