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Short communication

# Stimulus dependence of non-steroidal antiinflammatory drug potency in a cellular assay of prostaglandin H synthase-2

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**Abstract**

The prostaglandin H synthase-2 selective non-steroidal antiinflammatory drugs nimesulide, NS-398 (*N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide), flosulide and SC 58125 (5-(4-fluorophenyl)-1-[4-(methylsulfonyl)phenyl]-3-(trifluoromethyl)-1*H*-pyrazole) as well as the non-selective non-steroidal antiinflammatory drugs indomethacin, meclofenamate and ibuprofen were compared in a WISH (human amnionic epithelial cell) cellular assay of prostaglandin H synthase-2 activity. Varying amounts of prostaglandin $E_2$ were induced in WISH cells using either interleukin-1β, tumor necrosis factor-α or phorbol 12-myristate 13-acetate, alone or in combination, or with okadaic acid as stimulants. The results from these studies demonstrated that under conditions which generate greater amounts of prostaglandin $E_2$, the potency of both prostaglandin H synthase-2 selective and non-selective non-steroidal antiinflammatory drugs may be reduced. Dexamethasone, a transcriptional inhibitor of prostaglandin H synthase-2, also became progressively less effective in cells activated by combinations of stimuli or with okadaic acid. We conclude that decreases in potency under conditions of high levels of prostaglandin H synthase-2 expression and prostaglandin $E_2$ production are observed equally with prostaglandin H synthase-2 selective and non-selective inhibitors. © 1997 Elsevier Science B.V.

*Keywords:* Prostaglandin H synthase-2; Non-steroidal anti-inflammatory drug; WISH cell

## 1. Introduction

Prostaglandin H synthase-2 is induced in response to mitogenic and proinflammatory stimuli in a wide variety of cell types leading to significantly increased prostaglandin production (Herschman, 1996). Most of the non-steroidal antiinflammatory drugs currently in use inhibit both prostaglandin H synthase-2 as well as the constitutive prostaglandin H synthase isoform prostaglandin H synthase-1 which is involved in normal gastric and renal homeostatic functions (Vane and Botting, 1995). Recent research efforts have therefore been focused on the development and characterization of selective prostaglandin H synthase-2 inhibitors with the goal of sparing patients from the gastric damage and renal toxicity presently associated with current non-steroidal antiinflammatory drug therapy.

The first set of pharmacologic criteria that must be met when screening for selective prostaglandin H synthase-2 inhibitors is prostaglandin H synthase-2 specific potency in a panel of in vitro biochemical and cellular assays. Initially, investigators calculated selectivity ratios of non-steroidal antiinflammatory drugs from paired assays of cells derived from different species such as arachidonic acid stimulated bovine aortic endothelial cells for prostaglandin H synthase-1 activity and lipopolysaccharide stimulated J774 mouse macrophages as a measure of prostaglandin H synthase-2 activity (Mitchell et al., 1994). Early testing of non-steroidal antiinflammatory drug selectivity was also done using microsomal preparations of COS-1 cells expressing either murine prostaglandin H synthase-1 or prostaglandin H synthase-2 (Meade et al., 1992, 1993). Assays have since been established to determine human prostaglandin H synthase isoform selectivity using microsomal preparations of recombinant human prostaglandin H synthase-1 and -2 expressed in baculovirus (Barnett et al., 1994; Cromlish et al., 1994) and vaccinia virus (O'Neill et al., 1994), human whole blood assays of lipopolysaccharide vs. ionophore stimulated thromboxane production from the same donor (Patrignani et al., 1994), parallel whole cell assays using human recombinant prostaglandin H synthase-1 and -2 isoforms transfected into mammalian cell lines (Kargman et al., 1996) as well as the coupled use of

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human cell lines such as U937 for prostaglandin H synthase-1 and osteosarcoma cells for prostaglandin H synthase-2 (Chan et al., 1995). It has been recognized that absolute selectivity and potency of compounds for prostaglandin H synthase-2 can differ widely depending upon the assay and conditions used in testing and so it has become critical in the drug discovery process to examine inhibitor selectivity in several complementary systems.

In the present study, we describe a whole cell assay for prostaglandin H synthase-2 using the human amnionic epithelial line known as WISH cells (Hayflick, 1961). Our previous work demonstrated that WISH cells produce prostaglandin $E_2$ via the induction of prostaglandin H synthase-2 in response to okadaic acid, interleukin-1β, tumor necrosis factor-α, phorbol 12-myristate 13-acetate or combinations of phorbol 12-myristate 13-acetate with interleukin-1β or tumor necrosis factor-α and that the magnitude of prostaglandin $E_2$ produced is stimulus dependent (Hulkower et al., 1997). We decided to examine the effect of these different stimuli on the relative potency of representative non-steroidal antiinflammatory drugs to inhibit prostaglandin $E_2$ production in WISH cells. The present study compares the potencies of the non-selective non-steroidal antiinflammatory drugs indomethacin, meclofenamate and ibuprofen (Munroe and Lau, 1995) and the prostaglandin H synthase-2 selective compounds nimesulide (Ward and Brogden, 1988), NS-398 (*N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide) (Futaki et al., 1994), flosulide (Klein et al., 1994) and SC 58125 (5-(4-fluorophenyl)-1-[4-(methylsulfonyl)phenyl]-3-(trifluoromethyl)-1*H*-pyrazole) (Reitz et al., 1994) in WISH cells when alternate stimuli are used to induce prostaglandin $E_2$ production. This work also examines the effect of dexamethasone, a transcriptional inhibitor of prostaglandin H synthase-2 mRNA (Kujubu and Herschman, 1992), when alternate stimuli are used to induce prostaglandin H synthase-2 in WISH cells.

## 2. Materials and methods

### 2.1. Reagents

The following supplies were obtained from the indicated sources: WISH cells, ATCC (Rockville, MD, USA); cell culture products and media, Gibco (Grand Island, NY, USA); recombinant human interleukin-1β and tumor necrosis factor-α, UBI (Lake Placid, NY, USA); okadaic acid, sodium salt, RBI (Natick, MA, USA); dexamethasone, ibuprofen, indomethacin, meclofenamate and phorbol 12-myristate 13-acetate, Sigma (St. Louis, MO, USA); casein, BDH Laboratory Supplies (Poole, UK); purified prostaglandin H synthase-1, prostaglandin H synthase-2 and rabbit polyclonal anti-human prostaglandin H synthase-1 and prostaglandin H synthase-2, Cayman Chemicals (Ann Arbor, MI, USA); 10% polyacrylamide pre-cast gels, nitrocellulose membrane, molecular weight standards and electrophoresis/transfer buffers and equipment, Novex (San Diego, CA, USA); prostaglandin $E_2$ enzyme immunoassay reagents, PerSeptive Diagnostics (Cambridge, MA, USA); streptavidin horseradish peroxidase, enhanced chemiluminescence reagents and Hyperfilm, Amersham (Arlington Heights, IL, USA); biotinylated goat anti-rabbit $F(ab')_2$ fragments, Biosource International (Camarillo, CA, USA). The prostaglandin H synthase-2 selective inhibitors nimesulide, NS-398, flosulide and SC 58125 were synthesized in-house at Abbott Laboratories (Abbott Park, IL, USA). All other chemicals were obtained though standard suppliers.

### 2.2. Cellular assay

Human amnionic WISH cells (Hayflick, 1961) were maintained in Dulbecco's minimal essential medium supplemented with 10% fetal bovine serum and 1% antibiotics (final concentration 50 U/ml penicillin G sodium and 50 μg/ml streptomycin sulfate). Cells were passaged via trypsinization and seeded at a density of $1 \times 10^5$ cells/cm$^2$ growth area into 48-well culture dishes for activation studies or into 6-well culture dishes for immunoblot analysis. After 48 h (≥ 90% confluence), the growth medium was decanted and the monolayers were washed twice with Gey's balanced salt solution. The cells were then treated with 10 ng/ml of interleukin-1β, tumor necrosis factor-α or phorbol 12-myristate 13-acetate or with 30 nM okadaic acid in Neuman-Tytell serumless medium containing 1% penicillin-streptomycin solution. As indicated, indomethacin, meclofenamate, ibuprofen, nimesulide, NS-398, flosulide, SC 58125 and dexamethasone were added to the cultures at the time of treatment (final dimethyl sulphoxide concentration ≤ 0.1%). Following an 18 h incubation at 37°C in a humidified $CO_2$ incubator, the conditioned medium was removed from the cultures and assayed for prostaglandin $E_2$ content. Inhibition of prostaglandin $E_2$ production in WISH cells by the non-steroidal antiinflammatory drugs and dexamethasone was calculated by comparison of the levels of prostaglandin $E_2$ produced by drug treated cultures with that of vehicle treated controls for each stimulus used. The $IC_{50}$ values and 95% confidence limits were computed using linear regression analysis on data that fell on the linear portion of the percent inhibition versus $\log_{10}$ inhibitor concentration curves. Each experiment was repeated at least three times with each data point in triplicate.

### 2.3. Prostaglandin $E_2$ assay

The prostaglandin $E_2$ content of the conditioned media was assayed using the PerSeptive Diagnostics enzyme immunoassay kit according to the manufacturer's protocol. The anti-prostaglandin $E_2$ antibody has < 3.5% cross-reactivity with prostaglandin $A_1$, prostaglandin $A_2$, prosta-

glandin $B_1$, prostaglandin $B_2$, prostaglandin $F_{1\alpha}$ and prostaglandin $F_{2\alpha}$. Sensitivity of this enzyme immunoassay is 15 pg/ml with a detection range of 0.1–50 ng/ml.

### *2.4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot analysis*

WISH cells treated in 6-well culture dishes were solubilized directly into Laemmli sample buffer and stored at −20°C for subsequent electrophoresis (Laemmli, 1970) and immunoblotting. The protein content of cell lysates was determined using the method of Bensadoun and Weinstein (1976) with bovine serum albumin as a standard. Samples (50 μg protein) of whole cell homogenates in Laemmli buffer were subjected to electrophoresis on 1.5 mm thick 10% polyacrylamide gels. The separated proteins were transferred onto nitrocellulose filters using half-strength Towbin buffer (Towbin et al., 1979) with 20% methanol (v/v). The filters were blocked for 1 h at room temperature with 2.5% casein in 50 mM Tris HCl (pH 7.6), 154 mM NaCl and 0.2 mM thimerosal and then incubated with anti-prostaglandin H synthase antibody (1:1000 dilution) in blot buffer (50 mM Tris HCl, 200 mM NaCl, 0.05% Tween-20 and 1% casein, pH 7.5) overnight at 4°C. Following 3 × 5 min washes in TBS/Tween (50 mM Tris HCl, 200 mM NaCl, pH 7.5, containing 0.05% Tween-20), the filters were incubated with biotinylated goat anti-rabbit $F(ab')_2$ fragments (1:2000 dilution) in blot buffer for 30 min at room temperature and then washed (3 × 5 min) in TBS/Tween. The filters were then incubated with streptavidin horseradish peroxidase (1:5000 dilution) in blot buffer for 30 min at room temperature, and then washed (3 × 5 min) in TBS/Tween. The immunoreactive bands were visualized using the enhanced chemiluminescence reagents followed by a 30 s exposure to Hyperfilm (Amersham) according to the manufacturer's protocol.

## 3. Results

WISH cells produce prostaglandin $E_2$ in response to stimulation with either interleukin-1β, tumor necrosis factor-α, phorbol 12-myristate 13-acetate or okadaic acid. The most abundant production of prostaglandin $E_2$ was observed using okadaic acid as a stimulus or with combinations of two stimuli such as interleukin-1β and tumor necrosis factor-α or phorbol 12-myristate 13-acetate with either interleukin-1β or tumor necrosis factor-α (Fig. 1A). In all cases, the level of prostaglandin $E_2$ produced upon stimulation correlated to the induction of prostaglandin H synthase-2 protein (Fig. 1B) and the levels of prostaglandin H synthase-1 protein were unaffected in response to stimulation as we had observed previously (Hulkower et al., 1997).

The results of the studies using the structurally diverse,

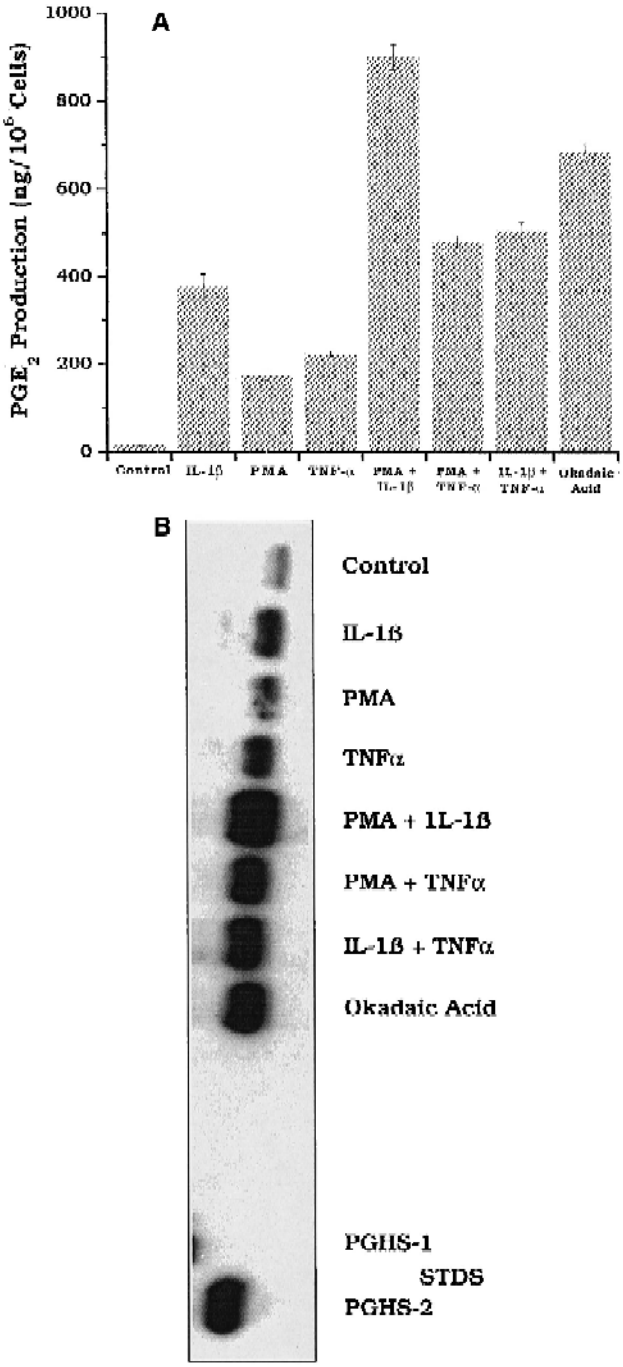


Fig. 1. Effect of stimulus on prostaglandin $E_2$ production and prostaglandin H synthase-2 induction by human amnionic WISH cells. (A) WISH cells in 48-well plates were treated for 18 h with 30 nM okadaic acid, 10 ng phorbol 12-myristate 13-acetate/ml, 10 ng interleukin-1β/ml, 10 ng tumor necrosis factor-α/ml and combinations of phorbol 12-myristate 13-acetate, interleukin-1β and tumor necrosis factor-α as noted and the amount of prostaglandin $E_2$ released into the conditioned medium was measured by specific enzyme immunoassay. Values shown are the mean ± S.E.M. of 6 determinations. (B) Immunoblot analysis using a 1:1000 dilution of anti-human prostaglandin H synthase-2 IgG of whole cell homogenates (50 μg protein) from corresponding 6-well plate cultures treated for 18 h as described in panel A. The lanes marked prostaglandin H synthase-1 and prostaglandin H synthase-2 indicate purified ovine prostaglandin H synthase proteins run as standards.

non-selective non-steroidal antiinflammatory drugs indomethacin, meclofenamate and ibuprofen are summarized in Table 1. Indomethacin was equipotent in its ability to inhibit prostaglandin $E_2$ in WISH cells with overlapping $IC_{50}$ values in the 10–20 nM range regardless of stimulus. Similar findings were made with only one exception for meclofenamate. $IC_{50}$ values for meclofenamate, which was the most potent compound tested in this study, overlapped between 0.3–1.0 nM for all stimuli with the exception of the combination of phorbol 12-myristate 13-acetate and interleukin-1 where the potency dropped by nearly 3-fold to an $IC_{50}$ of 2.9 nM. Ibuprofen, known to be a competitive inhibitor of prostaglandin H synthase-1, was by far the least potent of the non-steroidal antiinflammatory drugs tested in this study and showed more pronounced stimulus dependent differences in potency. Ibuprofen had $IC_{50}$ values of 150–200 nM when phorbol 12-myristate 13-acetate, interleukin-1 and tumor necrosis factor were used as single agents to induce prostaglandin $E_2$ production in WISH cells. However, the potency of ibuprofen showed a dramatic decrease when either a combination of activators or okadaic acid was used to stimulate prostaglandin $E_2$ production in WISH cells. Stimulation with the combination of interleukin-1 with either tumor necrosis factor or phorbol 12-myristate 13-acetate caused the potency of ibuprofen to drop by 3-fold to give $IC_{50}$ values in the 600 nM range. A 5-fold drop in potency to an $IC_{50}$ of nearly 1 μM was observed with this compound when okadaic acid was used as the stimulus. Thus while indomethacin, meclofenamate and ibuprofen are all non-selective non-steroidal antiinflammatory drugs, differences can be inferred about their mechanisms of inhibition of the prostaglandin H synthase-2 enzyme by measuring their potency under different conditions of cellular activation.

Results of similar studies using the prostaglandin H synthase-2 selective inhibitors nimesulide, NS-398, flosulide and SC 58125 are also summarized in Table 1. Nimesulide gave overlapping $IC_{50}$ values of 9–16 nM against prostaglandin $E_2$ production by WISH cells stimulated under all conditions except for okadaic acid and the combination of phorbol 12-myristate 13-acetate and interleukin-1 where it was 2- to 4-fold less potent with an $IC_{50}$ of 36 nM. NS-398 was slightly more potent than nimesulide having $IC_{50}$ values in the 3–5 nM range against prostaglandin $E_2$ production induced by interleukin-1, phorbol 12-myristate 13-acetate and the combination of interleukin-1 with either phorbol 12-myristate 13-acetate or tumor necrosis factor. As observed for nimesulide, NS-398 was also less potent against WISH cells stimulated with either okadaic acid or the combination of phorbol 12-myristate 13-acetate and interleukin-1 ($IC_{50}$ values of 15 and 19 nM respectively). Interestingly, NS-398 was at least 10-fold more potent with an $IC_{50}$ of $< 0.5$ nM against tumor necrosis factor induced prostaglandin $E_2$ production as opposed to its potency in cells stimulated by any other activators. Flosulide gave three discrete sets of $IC_{50}$ values which were distinctly associated with particular stimulation conditions used in the assay. Flosulide was the most potent against prostaglandin $E_2$ production in WISH cells induced by either phorbol 12-myristate 13-acetate, tumor necrosis factor or interleukin-1 alone with overlapping $IC_{50}$ values of 13–25 nM. Flosulide was approximately 3-fold less potent ($IC_{50}$ values of 70–86 nM) when phorbol 12-myristate 13-acetate, interleukin-1 and tumor necrosis factor were used in combination to induce prostaglandin $E_2$ production in WISH cells. Another marked reduction in the potency of flosulide was noted when okadaic acid was used as the stimulus in

Table 1
Effect of stimulus on the potency of non-steroidal antiinflammatory drugs and dexamethasone against prostaglandin $E_2$ production by WISH cells

| Drug | IL-1β | TNF-α | PMA | IL-1 + TNF | PMA + TNF | PMA + IL-1 | OKA |
|---|---|---|---|---|---|---|---|
| Indomethacin | 11.8 (7.4–17.4) | 21.3 (16.7–27.9) | 7.0 (3.2–11.5) | 13.4 (9.8–17.8) | 10.8 (8.1–14.1) | 10.5 (7.7–13.5) | 12.8 (9.4–17.0) |
| Meclofenamate | 0.69 (0.44–1.2) | 0.62 (0.39–1.0) | 0.25 (0.20–0.30) | 0.66 (0.55–0.79) | 0.53 (0.33–0.83) | 2.9 (2.0–4.7) | 0.96 (0.78–1.2) |
| Ibuprofen | 208.0 (153.3–275.5) | 177.1 (113.7–259.2) | 150.0 (75.5–253.3) | 584.2 (471.5–726.5) | 308.0 (233.0–406.7) | 645.6 (572.0–741.0) | 964.2 (796.3–1196.0) |
| Nimesulide | 11.4 (7.93–17.1) | 14.8 (12.3–17.8) | 8.9 (7.4–10.8) | 10.3 (7.5–13.7) | 16.4 (11.2–24.4) | 35.7 (29.7–40.7) | 35.6 (25.7–47.0) |
| NS-398 | 2.9 (2.3–3.5) | 0.41 (0.29–0.56) | 3.1 (2.2–4.1) | 5.0 (3.9–6.3) | 5.0 (3.9–6.2) | 19.3 (16.2–23.2) | 15.1 (9.6–23.0) |
| Flosulide | 24.7 (19.1–31.7) | 17.8 (11.2–26.6) | 12.5 (4.7–23.8) | 77.8 (60.1–103.7) | 69.7 (49.2–106.2) | 85.9 (62.8–124.2) | 185.8 (168.2–203.2) |
| SC 58125 | 52.7 (33.7–76.6) | 43.9 (20.8–76.0) | 41.3 (21.2–67.9) | 112.8 (80.7–150.6) | 77.8 (66.1–91.1) | 324.7 (285.3–373.5) | 259.3 (216.7–315.1) |
| Dexamethasone | 0.57 (0.40–0.78) | 0.44 (0.37–0.53) | 0.67 (0.55–0.80) | 2.6 (1.1–5.5) | 2.3 (1.4–3.3) | 21.7 (12.1–50.7) | > 10 μM |

WISH cells were stimulated for 18 h with 30 nM okadaic acid (OKA), 10 ng phorbol 12-myristate 13-acetate/ml (PMA), 10 ng interleukin-1β/ml (IL-1), 10 ng tumor necrosis factor-α/ml (TNF) and combinations of phorbol 12-myristate 13-acetate, interleukin-1β and tumor necrosis factor-α and the indicated drugs as noted. The $IC_{50}$ values against prostaglandin $E_2$ production by WISH cells are shown in nM with the corresponding 95% confidence limits given below in parentheses.

WISH cells ($IC_{50}$ of 186 nM). SC 58125 showed a similar drop in potency against either okadaic acid or phorbol 12-myristate 13-acetate and interleukin-1 induced prostaglandin $E_2$ production with $IC_{50}$ values of 259 and 325 nM respectively as compared to overlapping $IC_{50}$ values of 40–80 nM for prostaglandin $E_2$ production induced by single activators or by the combination of phorbol 12-myristate 13-acetate and tumor necrosis factor. SC 58125 had an intermediate $IC_{50}$ of 113 nM against prostaglandin $E_2$ production induced by the combination of interleukin-1 and tumor necrosis factor and overall was the least potent of the prostaglandin H synthase-2 selective non-steroidal antiinflammatory drugs tested in WISH cells.

The steroid dexamethasone which acts as a transcriptional inhibitor of the prostaglandin H synthase-2 gene (Kujubu and Herschman, 1992) gave $IC_{50}$ values of $< 1$ nM against individual stimulators of prostaglandin $E_2$ production (range of 0.4–0.7 nM). Dexamethasone still retained significant potency ($IC_{50}$ values of 2–3 nM) against prostaglandin $E_2$ production induced by the combination of tumor necrosis factor with either interleukin-1 or phorbol 12-myristate 13-acetate while it was 10-fold less potent ($IC_{50}$ of 22 nM) against prostaglandin $E_2$ production induced by the combination of phorbol 12-myristate 13-acetate and interleukin-1 (Table 1). As we have noted previously, when prostaglandin $E_2$ was induced in WISH cells using the combination of phorbol 12-myristate 13-acetate and interleukin-1 we were unable to obtain inhibition by dexamethasone of $> 75\%$ even with high concentrations of this compound. Interestingly, we were unable to calculate a valid $IC_{50}$ for dexamethasone against okadaic acid induced prostaglandin $E_2$ production as the inhibition achieved rarely surpassed 55% even at concentrations of dexamethasone up to 10 $\mu$M.

## 4. Discussion

WISH cells have the capacity to produce robust quantities of prostaglandin $E_2$ through the upregulation of prostaglandin H synthase-2 by a variety of stimuli. We have developed a cellular assay of prostaglandin H synthase-2 activity which can be variably regulated in order to approximate the extent to which cells may be activated depending upon the mixture and type of cytokines found at physiological sites of inflammation. This work demonstrates that by altering the conditions for cellular activation, the potency of both prostaglandin H synthase-2 selective and non-selective non-steroidal antiinflammatory drugs against prostaglandin $E_2$ production may be significantly modified within a given cell.

In cell-free preparations of enzymes, prostaglandin H synthase-2 selective non-steroidal antiinflammatory drugs such as NS-398 appear to act as time-dependent, irreversible inhibitors of prostaglandin H synthase-2 while they are time-independent, reversible inhibitors of prostaglandin H synthase-1 (Copeland et al., 1994; Ouellet and Percival, 1995). On the other hand, non-selective non-steroidal antiinflammatory drugs such as indomethacin are time-dependent, irreversible inhibitors of both prostaglandin H synthase-1 and prostaglandin H synthase-2 (Copeland et al., 1994; Ouellet and Percival, 1995). Non-steroidal antiinflammatory drugs such as ibuprofen may also act as reversible, competitive inhibitors of arachidonic acid substrate (Kargman et al., 1996). It has been shown recently that the potency and selectivity of certain non-steroidal antiinflammatory drugs in assays using prostaglandin H synthase transfected CHO cell lines can be affected also by substrate concentration from either endogenously liberated sources or by exogenously added arachidonate (Kargman et al., 1996). Indeed in many whole cell systems, the upregulation of prostaglandin H synthase-2 protein occurs concomitantly with an increase in phospholipase $A_2$ enzymatic activity and protein as was previously demonstrated in rheumatoid synovial fibroblasts as well as in WISH cells (Hulkower et al., 1994; Xue et al., 1996). As our results show a trend towards an overall decrease in non-steroidal antiinflammatory drug potency under conditions where the most prostaglandin H synthase-2 and prostaglandin $E_2$ production is present, a possible explanation for this may be that endogenous arachidonic acid release from these cells could also be greatly increased under those same conditions and could therefore compete with the non-steroidal antiinflammatory drug for prostaglandin H synthase-2 binding. As activity of the inducible 85 kDa cytosolic phospholipase $A_2$ has been shown to be regulated by phosphorylation by both mitogen activated protein kinase (Lin et al., 1993) as well as other protein kinases (DeCarvalho et al., 1996), it is conceivable that the different sets of stimuli could differentially modulate arachidonic acid release in WISH cells by affecting both the abundance and phosphorylation state of cytosolic phospholipase $A_2$. Okadaic acid has been shown to increase the level of phosphorylation of serine residues of cytosolic phospholipase $A_2$ as well as to increase arachidonic acid release from human monocytes (DeCarvalho et al., 1996). Another possibility is that the additional mass of prostaglandin H synthase-2 enzyme which is induced by the more robust stimuli would require larger quantities of inhibitor to quench its activity, i.e. competitive inhibition. The notable exceptions to this are indomethacin and meclofenamate which we have found to be nearly equipotent under all activation conditions in WISH cells.

It is interesting that the potency of dexamethasone, a transcriptional inhibitor of prostaglandin H synthase-2 (Kujubu and Herschman, 1992), is also diminished when either a combination of agents or okadaic acid is used to stimulate WISH cells. Okadaic acid, a phosphatase inhibitor, may prevent the dephosphorylation of IκB, the inhibitor synthesized in response to steroids which prevents the activation of NFκB (Scheinman et al., 1995). Thus, the newly synthesized IκB would remain phospho-

rylated due to okadaic acid treatment and as such would remain vulnerable to degradation (Menon et al., 1995) allowing some transcription of the prostaglandin H synthase-2 gene to occur through NFκB even in the presence of high levels of dexamethasone. It is possible that okadaic acid could also serve to prolong the half life of prostaglandin H synthase-2 mRNA in a similar fashion to that observed in cells treated with the combination of phorbol 12-myristate 13-acetate and interleukin-1 (Ristimaki et al., 1994). Indeed, our findings indicate that significantly more dexamethasone is required to inhibit prostaglandin $E_2$ production induced in response to the combination of phorbol 12-myristate 13-acetate and interleukin-1 than with each of these stimuli separately (Table 1). Although these observations have been made in vitro, these studies raise the possibility that perhaps there are also disease states which might be steroid resistant. It should be noted that besides dexamethasone, none of the other compounds tested affected the induction of prostaglandin H synthase-2 protein in WISH cells (data not shown).

The assay described in this study may be a useful way to mimic the production of prostaglandin $E_2$ at progressively different levels of inflammation in an in vitro setting and to assess the potency of a given inhibitor in a single cell type when activation conditions are varied. Our findings would suggest that there are two or possibly three such types of inhibitory environments that an inhibitor could encounter depending upon the activation state of the cell. One such environment is found when the cells are activated by a single stimulus such as phorbol 12-myristate 13-acetate, tumor necrosis factor or interleukin-1, a second environment is found when combinations of these stimuli are used and perhaps a third type of environment is induced by okadaic acid. Prostaglandin H synthase-2 selective inhibitors do not appear to offer any particular advantage in general over non-selective compounds in resisting decreases in efficacy under conditions of high levels of prostaglandin H synthase-2 expression and prostaglandin $E_2$ production.

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