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EFFECT OF BENZYDAMINE, A TOPICALLY ADMINISTERED NSAID, ON *IN VITRO* PROSTANOID SYNTHESIS BY HUMAN AND RAT GASTRIC MUCOSA AND RAT KIDNEY, AORTA AND URINARY BLADDER: LACK OF EFFECT ON CYCLOOXYGENASE BUT POTENT INHIBITION OF RECEPTOR-LINKED PROSTANOID SYNTHESIS

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ABSTRACT

Jeremy JY, Kirk RM, Mikhailidis DP. Effect of benzydamine, a topically administered NSAID, on *in vitro* prostanoid synthesis by human and rat gastric mucosa and rat kidney, aorta and urinary bladder: lack of effect on cyclooxygenase but potent inhibition of receptor-linked prostanoid synthesis. Inflammopharmacology. 1991:1:151–159.

Orally administered NSAIDs are notorious for their frequent and severe side-effects in the gastrointestinal tract and kidney, whereas topically administered NSAIDs may avoid these untoward effects. Since NSAID-induced side-effects are largely prostaglandin (PG)-mediated, the effects of the topically administered NSAID, benzydamine, on *in vitro* PGI_2 and PGE_2 synthesis by the rat and human gastric mucosa and rat kidney slices was investigated. The effect on receptor-linked PG synthesis in the isolated rat aorta (adrenergic) and urinary bladder (cholinergic) was also investigated since NSAIDs may disrupt mobilization of calcium therein. Benzydamine was a very weak inhibitor of spontaneous PGI_2 and PGE_2 synthesis by human and rat gastric mucosa and rat kidney. In contrast, benzydamine was a potent inhibitor of noradrenaline- and carbachol-stimulated (but not arachidonate- or trauma-stimulated) PGI_2 synthesis. It is concluded that: a) benzydamine is unlikely to elicit cyclooxygenase-mediated side-effects on the gastrointestinal tract or kidney, b) the anti-inflammatory action of benzydamine is mediated by disruption of calcium linked to receptor–PG synthesis coupling, and c) calcium-dependent inflammatory processes may also be affected by benzydamine.

Keywords: benzydamine, NSAID, prostaglandin, gastric mucosa, kidney, aorta, bladder

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are the principal drugs of choice in alleviating inflammation and pain associated with rheumatoid arthritis (RA), osteoarthritis (OA), gout and ankylosing spondylitis. However, NSAID administration is also associated with several adverse side-effects, principally ulceration, perforation and haemorrhage of the stomach and small intestine [1–3] and to a lesser extent with impaired renal function [4,5] and hypertension [5,6].

It has been suggested that NSAIDs induce gastrointestinal toxicity via two main mechanisms: a) inhibition of synthesis of the cytoprotective prostanoids, PGI_2 and PGE_2 [7,8], and b) direct physicochemical toxicity caused by high concentrations of

NSAIDs in the gastric lumen following oral administration [3,9]. Side-effects of NSAIDs on the kidney and vasculature are also thought to be mediated by endogenous PG inhibition from circulating NSAIDs [4–6]. One possible means of avoiding NSAID-induced side-effects is the use of topically administered anti-inflammatory drugs at the site of inflammation/pain. Since this type of drug is absorbed through the skin and at a slower rate than via gastrointestinal absorption, the transient high intragastric and systemic concentrations of the drug may theoretically be avoided. In turn, this may reduce the incidence of disturbances of the gastrointestinal tract, kidney and blood vessels associated with other orally administered NSAIDs.

In order to further explore this possibility, the effect of benzydamine (a novel topically administered NSAID), on *in vitro* PG synthesis by rat and human gastric mucosa was investigated. Since systemic NSAIDs are nephrotoxic in some cases, the effect of benzydamine on rat kidney was also studied. Recent studies have also demonstrated that NSAIDs may inhibit PG synthesis, not only via inhibition of cyclo-oxygenase (the enzyme complex that transforms arachidonic acid to endoperoxides, the immediate precursors of PG synthesis) but also through disruption of receptor-linked calcium mobilization [10,11] associated with activation of phospholipase A_2 (the enzyme that liberates arachidonate from phospholipid stores) [12]. In order to investigate possible effects on Ca^{2+}-linked PG synthesis, the effect of benzydamine on receptor-linked PG synthesis was also studied using two well-established systems: adrenoceptor-linked PG synthesis in the rat aorta [13] and muscarine receptor-linked PG synthesis in the rat urinary bladder [14]. In all studies, benzydamine was compared with naproxen.

MATERIALS AND METHODS

Materials

Benzydamine was supplied by 3M Riker (Loughborough, Leics, UK). Antisera against 6-oxo-$PGF_{1\alpha}$ and PGE_2 of high serological specificity were purchased from Capell Laboratories (West Chester, PA, USA). [^{3}H]-ligands were purchased from New England Nuclear (Dreieich, West Germany) and unlabelled ligands from Upjohn Co. (Kalamazoo, MI, USA). All other materials were purchased from Sigma (Poole, Dorset, UK).

Preparation and incubation of gastric mucosa and renal cortex

Human stomach tissue was obtained from six patients undergoing gastrectomy for carcinoma of the stomach. Only tissues with no obvious invasive neoplasm were used for the study, samples being checked by histology. Mucosa was dissected away from the muscularis and 5 mm discs cut using an ophthalmologist's trephine and the discs placed in pre-gassed (95% O_2:5% CO_2) Dulbecco's Minimum Essential Medium (MEM). Sprague Dawley rats (250 g) were decapitated and their stomachs (as well as aortae, kidneys, and urinary bladders; see below) excised. The rat stomachs were opened by longitudinal incision and discs (5 mm diameter) cut out on a cork board

using an opthalmologist's trephine, the mucosa dissected from the muscularis and the mucosal discs pooled and washed in MEM. Kidneys were cut longitudinally into two halves and the cortex dissected from the medulla. The medulla was discarded and the cortex cut into approximately 2 mm cubes. For both human and rat mucosal tissue, one disc, in duplicate, was placed in MEM containing varying concentrations of benzydamine and naproxen and incubated for 15 min at 37°C to allow equilibrium of the NSAIDs with the tissue. Naproxen was dissolved initially in absolute ethanol at a concentration of 100 mg/ml prior to dilution in MEM. The final concentrations of ethanol present in incubation media have no effect on PG synthesis (spontaneous or receptor-linked). Benzydamine is freely soluble in aqueous media. The buffer was then aspirated and discarded and 1 ml MEM containing the same concentrations of NSAIDs as in the preincubation phase added to the tissue. The tissue was then incubated at 37°C for 2 h. In preliminary experiments, it was established that this time course was optimal for the quantity and reproducibility of PG output by the tissues studied. The incubation tubes were centrifuged (15 min at 850 *g*) and aliquots of supernatant taken for estimation of 6-oxo-$PGF_{1\alpha}$ (the spontaneous, stable hydrolysate of PGI_2) and PGE_2 concentrations by radioimmunoassay, as previously described [15]. Two segments of kidney, in duplicate, were also placed in 1 ml MEM containing varying concentrations and processed for assessment of PG release as above for the gastric mucosa.

Preparation and incubation of aortic and urinary bladder tissue

Having removed adventitia and fatty tissue the aortae were cut into 2 mm rings with a scalpel blade on a Teflon block. The bladders were cut longitudinally into four strips and each strip into four further segments. Tissues were then pooled and randomized in MEM (pre-gassed with 95% O_2:5% CO_2) and incubated for 6 h in MEM, at 37°C, with changes of medium every 30 min, to allow prostanoid release elicited by preparative handling to subside [13,14]. Since PGI_2 is quantitatively the dominant prostanoid in both rat aorta and rat urinary bladder [12], concentration response curves were established using the measurement of 6-oxo-$PGF_{1\alpha}$ (the stable spontaneous hydrolysate of PGI_2) as an endpoint. Following preincubation as described above, tissue was placed in 1 ml MEM containing varying concentrations of NSAIDs (per tube: 3 aortic rings, or 2 bladder segments, in sextuplicate for each concentration of NSAID). The tissues were then incubated for 1 h at 37°C to allow equilibration with the NSAIDs. The medium was aspirated and replaced with fresh medium containing the same concentrations of NSAIDs. Prostanoid synthesis was then elicited in aortic tissue by the addition of 10 μmol noradrenaline and 30 μmol AA and in the bladder with 10 μmol carbachol and 10 μmol AA. Tissues were then further incubated for 1 h at 37°C and aliquots of supernatant taken for estimation of PG concentrations by radioimmunoassay [13,14]. Trauma-stimulated PG synthesis was elicited by both freeze fracturing and sonication of aortic and bladder tissue. Tissues were sequestered into tubes and pre-equilibrated with NSAIDs as above. Freeze thawing: the media was then aspirated and tubes placed in a freezer at −70°C for 1 h after which time medium containing the same concentrations of NSAIDs was added and the tissues incubated for 1 h at 37°C. 6-oxo-$PGF_{1\alpha}$ concentrations were measured in the supernatant, as above.

Treatment of results

Data on NSAID concentration–response curves are expressed graphically as percentage inhibition of stimulated prostanoid synthesis (mean ± SEM). Basal (non-stimulated) prostanoid concentrations were subtracted from stimulated values to give specific stimulated values (arbitrarily, zero % inhibition). The inhibition of prostanoid synthesis for each NSAID was calculated from this basal value. 100% inhibition is equivalent to basal output. IC_{50}s are defined as the concentration of NSAID required to inhibit prostanoid synthesis by 50%. Actual PG levels generated are also given in the figure legends.

RESULTS

At concentrations up to 10^{-2} mol/L, benzydamine had little effect on the spontaneous generation of either PGE_2 or PGI_2 by rat or human gastric mucosa or rat kidney. In contrast, naproxen inhibited the generation of these prostanoids by the tissues in a concentration-dependent manner (Figures 1–3). In the rat aorta, benzydamine inhibited noradrenaline-stimulated PGI_2 synthesis in a concentration-dependent manner, whereas benzydamine had little effect on PGI_2 synthesis elicited by freeze fracturing or arachidonate (Figure 4). In the urinary bladder, carbachol-stimulated PGI_2 synthesis was inhibited at concentrations of benzydamine (Figure 5) similar to

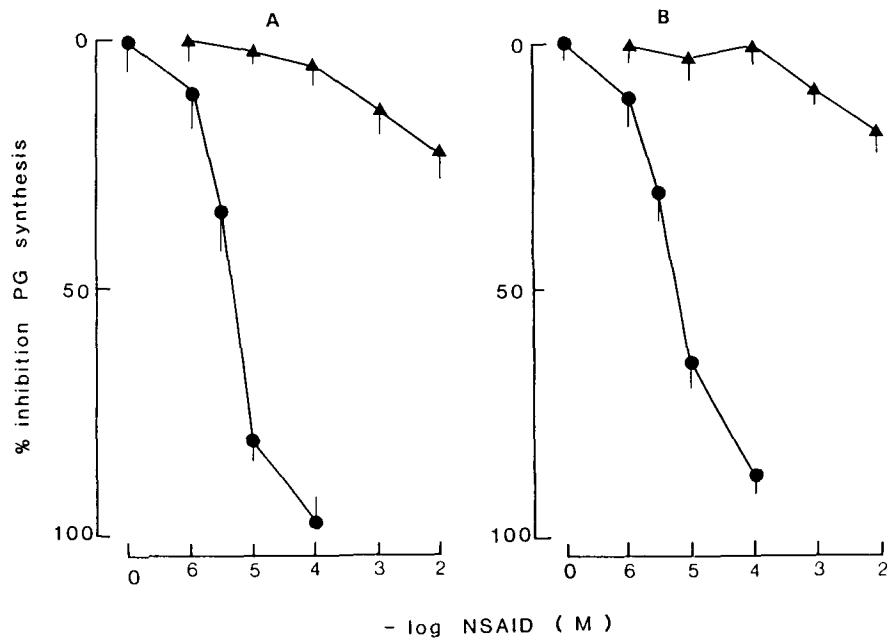

Figure 1. Effect of benzydamine (▲) and naproxen (●) on spontaneous PGI_2 (A) and PGE_2 (B) synthesis by human gastric mucosa. Each point represents mean ± SEM; $n=6$. IC_{50}s in mol/L: naproxen, $PGI_2 = 4.8 \times 10^{-6}$; benzydamine, PGI_2; $>1 \times 10^{-2}$. naproxen, $PGE_2 = 6 \times 10^{-6}$; benzydamine, $PGI_2 > 1 \times 10^{-2}$. Basal output (pg prostanoid (mg tissue)$^{-1}$ h^{-1}; 0%): PGI_2 (as 6-oxo-$PGF_{1\alpha}$) = 886, PGE_2 = 624

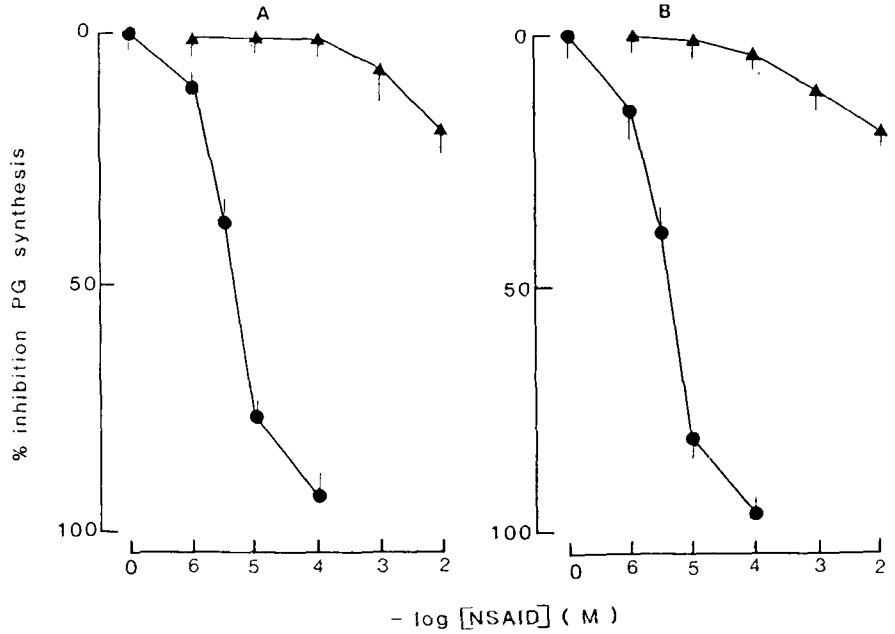

Figure 2. Effect of benzydamine (▲) and naproxen (●) on spontaneous PGI_2 (A) and PGE_2 (B) synthesis by rat gastric mucosa. Each point represents mean ± SEM; $n=6$. IC_{50}s in mol/L: naproxen, $PGI_2 = 4.8 \times 10^{-6}$; benzydamine, PGI_2; 1×10^{-2}; naproxen, $PGE_2 = 5.6 \times 10^{-6}$; benzydamine, $PGI_2 > 1 \times 10^{-2}$. Basal output (pg prostaglandin (mg tissue)$^{-1}$ h^{-1}; 0%) PGI_2 (as 6-oxo-$PGF_{1\alpha}$) = 784, PGE_2 = 343

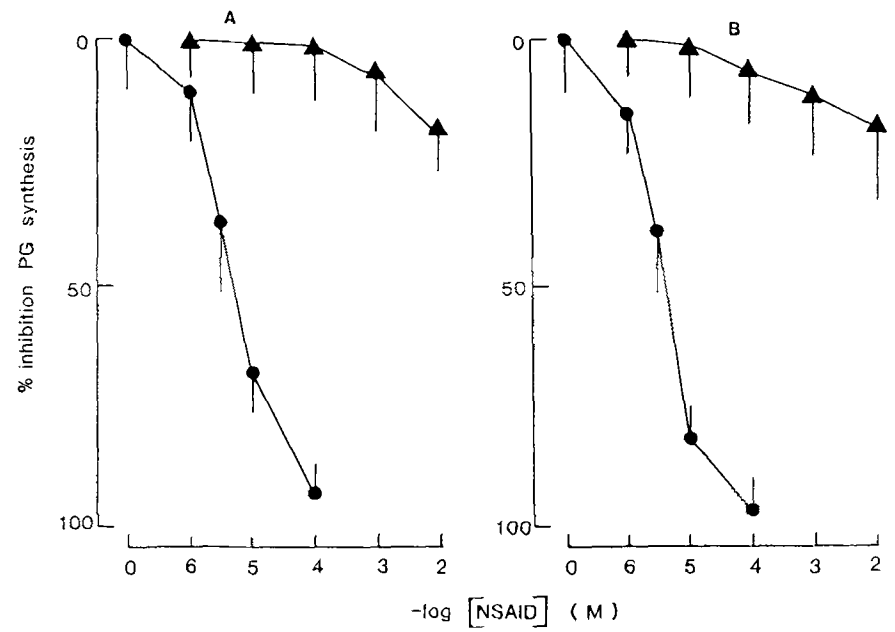

Figure 3. Effect of benzydamine (▲) and naproxen (●) on spontaneous PGI_2 (A) and PGE_2 (B) synthesis by rat renal cortex. Each point represents mean ± SEM; $n=6$. IC_{50}s in mol/L: naproxen, $PGI_2 = 4.9 \times 10^{-6}$; benzydamine, $PGI_2 > 1 \times 10^{-2}$; naproxen, $PGE_2 = 5.5 \times 10^{-6}$; benzydamine, $PGI_2 > 1 \times 10^{-2}$. Basal output (pg prostanoid (mg tissue)$^{-1}$ h^{-1}; 0%): PGI_2 (as 6-oxo-$PGF_{1\alpha}$) = 78, PGE_2 = 86

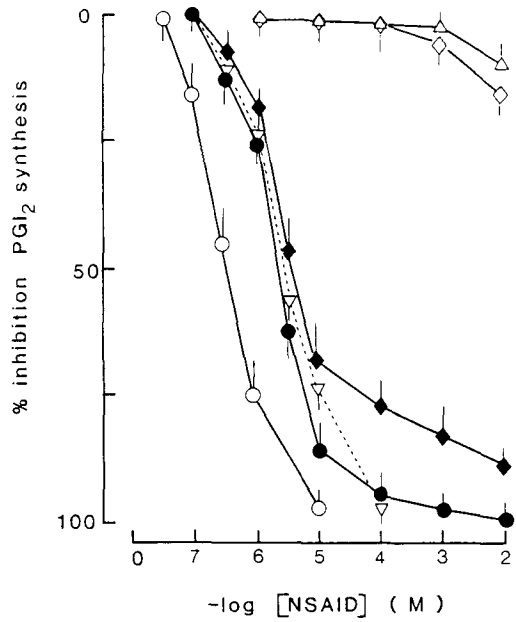

Figure 4. Effect of benzydamine on rat aortic PGI_2 synthesis when stimulated by noradrenaline (♦), freeze fracturing (△), and arachidonate (◊) and naproxen when stimulated with noradrenaline (○), freeze fracturing (▽) and arachidonate (●). Each point represents mean ± SEM; $n=6$. IC_{50}s (in mol/L): Benzydamine: freeze fracturing/arachidonate > 10^{-2}, noradrenaline-stimulated, 4.2×10^{-6}; Naproxen: freeze fracturing 2.9×10^{-6}, arachidonate, 1.7×10^{-6}, noradrenaline 3.3×10^{-7}.

noradrenaline-stimulated PGI_2 release by the aorta. Again benzydamine had little effect on PGI_2 synthesis elicited by freeze fracturing or arachidonate (Figure 5). IC_{50}s are given in the Figure legends.

DISCUSSION

The present study demonstrates firstly that benzydamine, compared with naproxen, is a very weak inhibitor (if at all) of spontaneous PG synthesis and release by the human and rat gastric mucosa and rat kidney. These findings are in general agreement with other studies [16,17] which showed benzydamine to inhibit PG synthesis in microsomes at very high concentrations (10^{-3} - 10^{-2} mol/L). Thus, it is unlikely that benzydamine at the concentrations reported [18] to be present in plasma following topical administration (2–150 ng/ml) would inhibit PG synthesis by the gastric mucosa or kidney. Consequently, it would be reasonable to predict that cyclo-oxygenase-mediated side-effects in these tissues would be minimal with this drug, following topical administration. This conclusion is in keeping with the lack of reports of side-effects on these organs when benzydamine is applied locally.

It is widely accepted that NSAID action is mediated principally by the inhibition of PGs at inflamed sites [19]. Since benzydamine has been proven effective in reducing inflammation and alleviating pain in both animal models and man [16,20–23], the

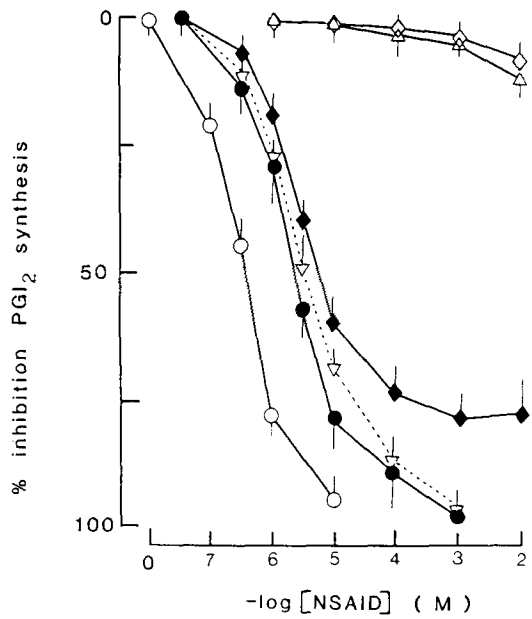

Figure 5. Effect of benzydamine on rat urinary bladder PGI_2 synthesis when stimulated by carbachol (♦), freeze fracturing (△), and arachidonate (◊) and naproxen on PGI_2 synthesis when stimulated with carbachol (○), freeze fracturing (●) and arachidonate (▽). Each point represents mean ± SEM; $n=6$. IC_{50}s (in mol/L): Benzydamine: freeze fracturing/arachidonate > 10^{-2}, carbachol-stimulated 7.3×10^{-6}; Naproxen: freeze fracturing 2.25×10^{-6}, arachidonate 3.7×10^{-6}, carbachol 2.85×10^{-7}.

question arises as to how benzydamine actually exerts its anti-inflammatory and analgesic action. The likely answer comes from the current experiments on receptor-linked PG synthesis in the rat aorta and urinary bladder. It was found that benzydamine was a weak inhibitor of PGI_2 synthesis in the rat aorta and bladder, when stimulated by AA or freeze fracturing, whereas at considerably lower concentrations (10^{-5} mol/L) benzydamine inhibited noradrenaline-stimulated and carbachol-stimulated PGI_2 release in the aorta and bladder, respectively. In a recent study it was demonstrated that indomethacin, ibuprofen and tiaprofenic acid inhibited receptor–PG synthesis coupling at lower concentrations than those required to inhibit AA- or trauma-stimulated PG synthesis in the aorta and bladder [10,11]. Both noradrenaline- and carbachol-stimulated PG synthesis in these tissues is linked to the mobilization of calcium (which in turn activates PLA_2 which liberates AA from endogenous stores) whereas trauma and arachidonate stimulate PG synthesis by bypassing this intrinsic receptor-linked calcium-mobilization step [12]. Thus, it is possible that benzydamine, as with other NSAIDs, inhibits the mobilization of calcium stores linked to PLA_2 activation. This conclusion is also in general agreement with Loffler *et al.* [24] who demonstrated that benzydamine inhibited the release of fatty acids from phospholipids at concentrations similar to those found to inhibit agonist-stimulated PG synthesis in this study.

Thus, although benzydamine appears to have little or no effect on cyclooxygenase, its relatively potent effect on receptor-linked PG synthesis may be of relevance to its analgesic and anti-inflammatory action via several mechanisms. It is well established that mediators of inflammation (histamine, serotonin, leukotrienes, interleukins, thromboxane A_2) are all potent stimulators of PG synthesis in a variety of tissues [25–30]. Given that inflammation involves the release of these mediators by cells such as leucocytes and mast cells, which in turn may elicit release of pro-inflammatory PGs at sites of inflammation, the anti-inflammatory action of benzydamine may be explained by blockade of these interactions. Furthermore, direct effects of benzydamine on calcium mobilization may explain the potency of this drug on other pro-inflammatory cells. For example, in studies on neutrophil function, benzydamine (10^{-4} to 10^{-6} mol/L) inhibits granule release, leucotaxis, adhesiveness to vessel walls and aggregation [16,31,32], all of which are calcium-dependent functions [33,34]. In this context, Abramson *et al.* [33] demonstrated that aspirin, indomethacin and piroxicam are also potent inhibitors of the early steps of receptor-activated neutrophil function, including the uptake and mobilization of calcium at the plasma membrane of these cells. A more recent study has also demonstrated that indomethacin and ibuprofen inhibits the uptake of calcium by human platelets [35]. It was proposed that NSAIDs may act by disrupting a 'trigger pool' of membrane calcium linked to signal transduction mechanisms [33–36]. These signal transduction mechanisms include the sequential activation of G proteins, phospholipase C and protein kinase C, all of which are modulated by calcium and ultimately determine the response of the neutrophil [33,34]. Furthermore, following topical administration, benzydamine has been found in synovial fluid at inflamed joints at concentrations [18] that would elicit inhibition of receptor-linked prostanoid synthesis as well as interfere with local white cell function.

In conclusion, at concentrations found in plasma, topically applied benzydamine is unlikely to elicit cyclooxygenase-mediated gastropathy, nephrotoxicity or hypertensive effects. The anti-inflammatory action of benzydamine is probably not due to inhibition of cyclooxygenase but possibly to disruption of calcium mobilization/sequestration linked to PG synthesis at the plasma membrane of inflammatory cells. Furthermore, since calcium is a universal second messenger, benzydamine may inhibit other calcium-dependent pro-inflammatory events (e.g. histamine release from mast cells). Such a possibility warrants further investigation.

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